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**The Effects of a Nutrient-Enriched Bar on Host Defense Mechanisms and Immunogenicity
of Hepatitis A Vaccine during US Army Ranger Training**

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13. ABSTRACT *(Maximum 200 words)*
This study was designed to assess the effects of a nutritionally-enhanced food bar and intense, protracted stress as engendered by US Army Ranger training on immune function as measured by immunogenicity to Hepatitis A vaccine, leukocyte phenotype, and delayed type hypersensitivity reaction. The stress of Ranger training caused a number of immunological changes from baseline, and the group consuming the bar fortified with antioxidants, indigestible carbohydrate, zinc, copper, selenium and other trace elements and vitamins manifested decreased effects on their immune function. For example, there was an attenuated reduction of the number of monocytes in the soldiers in the treatment vs. a placebo group with a likewise decreased reduction of T-lymphocytes in the treatment group. Other markers, such as body weight changes and numbers of B-lymphocytes, while not significant, indicated a clear trend toward beneficial effects in the treatment group. Hepatitis A vaccine was administered to both groups. The stress of Ranger training caused approximately a 30% reduction in antibody response when compared to a non-stressed group, but consumption of the nutritionally enriched bar had no significant effects on this response. Over 70% of those who completed this rigorous training regimen believed that supplementation with the bars, both treatment and placebo, helped them in completing Ranger training. While previous Ranger trainees had manifested significant weight loss during this training, the current class actually showed a weight gain. Generally, these results indicated that the experimental bars increased immune competence and status, contributed to a gain in weight during the training, and were very well tolerated and accepted by the training population.

14. SUBJECT TERMS
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EXECUTIVE SUMMARY

Vigorous Army training involves physical and psychological stress that causes immune dysregulation and increases risk of infection. Previous research had determined that a liquid product containing antioxidants, structured lipid (MCT/Canola), indigestible carbohydrate, vitamins, minerals, taurine, and carnitine blunted the immune suppression experienced by soldiers participating in the U.S. Army Special Forces Assessment and Selection School (21 days) (48). Ranger Training, a longer course (62 days), provided a unique opportunity to study the effects of a nutritional supplement on maintaining or minimizing the negative impact of stress on the immune system. A treatment food bar (approximately 65 grams in weight, similar in nutrient profile to the liquid product studied in SFAS) and a placebo bar (similar in macronutrient concentrations to the treatment product but lacking appreciable levels of key nutrients) were manufactured and studied during U.S. Army Ranger Training (RT). This study was designed to quantify the impact of intense, chronic stress on immune function (response to hepatitis A vaccine, leukocyte phenotype and functions, delayed-type hypersensitivity), body weight, cytokines, and hormones (cytokine and hormone data to be reported at a later date), and acceptance of this unique food bar during Ranger training.

One hundred ninety soldiers volunteered to participate in this double-blinded, randomized, placebo-controlled study. The subjects consumed two bars/day and blood collections were performed at four time points [Fort Benning, GA (baseline or visit one), Dahlonega, GA (mid-mountain and most intense stress or visit two), Dahlonega, GA (end-mountain or visit three), and Eglin, FL (end of study or visit four)] to assess the influence of physical and operational stress on immune response and determine if a unique nutritional formula provided in a food bar could maintain or minimize immunological changes. A group of soldiers that was similar in age but not undergoing training stress, received the vaccine and was used as a reference group.

We found that the stress of RT caused a number of immunologic changes and consumption of a formulated bar containing antioxidants, structured lipid, indigestible carbohydrate, zinc, copper, selenium and other vitamins and minerals attenuated the decrease in a number of measurements of immune function. The treatment resulted in a diminished decline in a number of clinically important immune cells. For example, there was an attenuated reduction in the number of monocytes in the treatment group as compared to the placebo group at the second ($P=0.041$) and third ($P=0.013$) time points. It was during the second and third time points when the intensity of the stress was most severe and where previous investigations had shown the greatest risk for and severity of infectious complications. At the last time point (visit four), total T-lymphocytes declined more significantly in the placebo group compared to those receiving the treatment bar (705 +/- 77 vs 443 +/- 62 cells/uL, $P<0.023$). This decline was the result of a decrease in the subset of CD4+ T-lymphocytes (helper/inducer) with a smaller decline occurring in the treatment group ($P=0.008$) at visit four (-482.5 +/- 51 cells/μL vs -300.4 +/- 39 cells/μL). Other significant changes included a difference in the Th1 lymphocyte subset and the activation of the lymphocytes as determined by the expression of the CD69 and CD40 ligands. Differences in other markers, e.g. body weight changes ($P = 0.068$) and B-lymphocytes ($P = 0.058$), although not statistically

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significant between the placebo and treatment groups, point to a benefit of the treatment bar. Unlike previous Ranger studies where appreciable weight loss was seen, in the current study both groups gained weight, primarily in the last phase. The cause of the weight gain is not clearly understood but the weight gain can not be completely attributed to the bars alone. Consequently, this factor may have made it more difficult to detect immunologic differences between the groups, and the results from this study may not be comparable to the nutritional and immunological observations of earlier studies. However, considering the extent of the immunological changes seen in this study, the conclusions of earlier studies may have over-emphasized the role of nutritional factors in the immune impairment.

One of the more compelling immune responses of this study was the depression in antibody response to a naive T-cell dependent vaccine. All soldiers were screened for prior hepatitis A vaccination or hepatitis A exposure. Fewer soldiers than anticipated were vaccinated in this study, due to previous exposure to hepatitis A virus or because of prior vaccination. Forty-three subjects in the placebo group (n=17 vaccinated) and 47 subjects in the treatment group (n=26 vaccinated) completed RT. There was no difference between the placebo and treatment groups in vaccine response at day 14 and 30 post vaccination; however, both groups experienced a 30% reduction in antibody response as compared to the non-stressed control group. Approximately 20% of the stressed soldiers did not achieve a sufficient level of antibody (seroconverion) to be considered "protected," whereas 100% of the non-stress group achieved a protected level. These results indicate that the severe stress of RT alters not only biomarkers of immune dysregulation, but also impairs functional markers of immune capacity (vaccine response).

INTRODUCTION

The training of Special Forces and Rangers involves physical and psychological stress as well as sleep deprivation. In the early 1990s the US Army Research Institute of Environmental Medicine (USARIEM) conducted studies of soldiers participating in Ranger Training (RT). It was found that soldiers lose more body weight and suffer relatively higher rates of infection as compared to soldiers undergoing less rigorous field training. Soldiers in RT had experienced a pneumonia outbreak (1992) and 4 deaths (1996) from hypothermia. These events initiated a series of studies assessing the nutritional and immunological impact of this stressful training and allowed the development of a Cooperative Research Agreement between Ross Products Division/Abbott Laboratories and USARIEM to study nutrient supplementation in both RT (62 days) and a shorter duration training program, Special Forces Assessment and Selection School (21 days). These training scenarios provide a unique environment to study nutritional supplements in a highly controlled, stressful environment. Furthermore, these studies allowed for the recruitment of many subjects in a short time period. The focus of this joint effort is to understand the mechanism by which individuals adapt (or fail to adapt) to physical and psychological stress affecting their performance, behavior and health. Our studies focused on how stress alters immunologic responses which may place soldiers at increased risk of infection or decrease the efficacy of vaccines administered during times of psychological and physical stress. Ross Products Division is interested in understanding the influence nutrient(s) may have on immune function, and RT provided an opportunity to evaluate them.

The original RT studies found an average daily energy expenditure of 4010 kcals/day. A daily energy deficit of approximately 1200 kcals/day caused loss of body weight (12% to 15%) in a short period of time (58). The physical stress was compounded by nutritional deprivation and immune dysregulation as measured by a high rate of skin DTH anergy among trainees (58). However, even with severe energy deficit, no measurable changes in vitamin or mineral levels could account for the immune dysregulation or increased rate of infections. In addition, no direct association between cellular immune function and clinical outcome (i.e. infection rate or vaccine response) was correlated positively as an endpoint of these early studies.

The Special Forces Assessment and Selection School (SFAS) stress is similar to RT while shorter in duration. We have studied nutritional formulations in soldiers participating in SFAS and investigated their effectiveness in minimizing the immune suppression that occurs from stress. We found that glutamine supplementation (15 grams/day) had no demonstrable effect on immune function (lymphocyte proliferation, leukocyte numbers, or delayed-type skin hypersensitivity-DTH). In a more recent study a combination antioxidant supplement (500 mg vitamin C, 400 IU of vitamin E, 15 mg β-carotene, and 100 μg selenium/day), attenuated the decreased lymphocyte proliferative response but did not influence DTH anergy or overall DTH induration. The most recent SFAS study by our group found that a ready-to-feed liquid product (formulated by Ross Products Division) containing vitamins and minerals with increased levels of antioxidants (compared to the previous study) plus taurine, carnitine, structured lipid (from canola oil and medium chain triglycerides), and indigestible carbohydrate (fructooligosaccharide)

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decreased the number of soldiers experiencing anergic DTH and increased the induration of the DTH response, compared to a placebo group (same energy content, without vitamins and minerals or the other special ingredients). Lymphocyte proliferation was increased in the treatment group whereas mean proliferative response decreased in the placebo group. In this study there was a positive effect of a nutritional supplement on a clinical endpoint in that the treatment group experienced fewer upper respiratory tract infections, compared to the placebo group $P < 0.05$). Positive results from this SFAS study prompted us to formulate a bar form (with similar nutrient profile as the ready-to-feed product) intended to be more easily inserted as part of the rations available during RT.

NUTRIENTS

In this study the experimental nutrition bars contained protein, fat and carbohydrate providing 14%, 30% and 56% of energy, respectively. The same amount of energy was included in the placebo bar to prevent any confounding factors. The bars contained similar proteins (high biologic value) from casein and soy and carbohydrates from honey, maltodextrin, and high fructose corn syrup. The treatment bar contained fructooligosaccharide (an indigestible carbohydrate added to maintain GI health) and a novel structured lipid made of medium chain triglycerides (6-8 carbon atoms) and canola oil [source of long chain fatty acids (LCFA), 16-18 carbon atoms]. Most dietary fats are triacylglycerol compounds with glycerol esters of fatty acids of 12-18 carbon atoms. This novel fat has random placement of the medium chain and long chain fatty acids on the glycerol backbone.

Structured Lipids

Fat digestion starts upon ingestion, with the majority of digestion and absorption occurring in the small intestine. Fat is released into the small intestine more slowly than water miscible substances. And fat, particularly triglycerides containing LCFA, are emulsified by bile salts. This dispersion occurs so that pancreatic lipase can hydrolyze the triacylglycerol into free fatty acids and 2-monoacylglycerol. The 2-monoacylglycerol is transported across the luminal cells and re-esterified for transport. Fat digestion and absorption changes markedly for MCT. These fats are digested, absorbed, and utilized in the absence of pancreatic lipase. Therefore, having medium chain fatty acid(s) (MCFA) as well as long chain fatty acid(s) (LCFA) on the same glycerol molecule we hoped to increase absorption and utilization of fatty acids as a muscle energy source thereby improving performance by minimizing catabolism of muscle tissue and muscle glycogen. Consumption of MCT's alone results in their absorption into the portal circulation resulting in a high level of first pass hepatic metabolism, whereas long chain triglycerides are absorbed and transported via the lymph entering the circulation prior to liver metabolism. Delivery to muscle tissue is increased where MCFA are oxidized preferentially over LCFA (75). In this study, we attempted to shift energy utilization away from muscle glycogen or protein and allow MCFA to be delivered via the structured lipid and utilized as a muscle energy source.

Carnitine/Taurine Supplementation Carnitine is required for lipid metabolism and is crucial

for energy flow within skeletal muscle. Trauma and infection lead to loss of carnitine (85). It has been shown that carnitine supplementation in rats decreased morbidity and mortality when challenged with lipopolysaccharide (LPS). Carnitine also improves deregulated lipid metabolism in septic and cachectic rats (90). There is evidence that carnitine may affect the immune system at several levels: 1) activation of phagocytes, especially granulocytes, 2) a role in specific phytohemagglutinin induced stimulation of T lymphocytes, and 3) a factor in membrane-activating effect on human NK cells (83).

Taurine (a β-amino-sulfonic acid), also included in the treatment bar, appears to be an effective scavenger of peroxidation products and is an important nutrient for immune function (89). Recently, Stapleton et al (78) reviewed taurine's influence on host defenses. They found that taurine plays a pivotal role in a number of physiologic functions ie. osmoregulation, antioxidation, detoxification and stimulation of glycolysis and glycogenesis, Taurine plays a specific role in neutrophil and macrophage function (72). Taurine constitutes greater than 50% of the free amino acids in lymphocytes (78). These cells seem to have a high dependence on β-amino acid transport and calcium flux (66). Furthermore, other types of stress or injury such as surgical injury (61), cancer (33) and sepsis (63, 86) have been correlated with decreased levels of plasma taurine.

Antioxidant Vitamins and Minerals

Strenuous exercise has been shown to increase production of oxygen free radicals (77); RT is demanding and highly stressful, both physically and mentally. If free radicals are produced without adequate metabolic control, harmful effects result. Immune cells such as lymphocytes, polymorphonuclear leukocytes and lymphoid tissues carry out many functions which generate free radicals. Ineffective elimination of free radicals can result in tissue injury, delayed healing, and increased susceptibility to infectious disease (23, 74). To control oxidation, immune cells contain concentrated amounts of antioxidants (59). Furthermore, immune cells function most efficiently when adequate levels of antioxidants are present (45).

β-carotene is an antioxidant and a vitamin A precursor, without the toxicity of other retinols (vitamin A) when given at high doses (4, 42). β-carotene is an effective antioxidant due to its ability to trap peroxyl radicals. It appears to be a unique antioxidant that is most effective at low atmospheric pressures (those found in capillaries and muscles) as well as efficient in quenching singlet oxygen species (9, 10). Gottlieb et al (30) found that β-carotene is effective in reducing lipid peroxidation [malondialdehyde (MDA)] in serum. Bendich (5) described several studies which demonstrated that β-carotene enhances immune function independent of its vitamin A activity. One relevant human study evaluated the effects of supplementation with 15-300 mg/day β-carotene on cell-mediated immune response as measured by skin DTH (47). When healthy male smokers were given 20 mg B-carotene for 14 weeks, there was a 12% higher proliferative response to mitogen phytohemagluttanin (PHA) than in a placebo-treated group. Watson et al. (87) found that 2 months of B-carotene (15 - 60 mg) supplementation enhanced the immune response in elderly subjects. *In vitro* culture of human PBMC with B-carotene induced alterations in cell phenotype and enhanced activation (70). Lastly, Kanter et al. (47) demonstrated that an antioxidant mixture (592 mg alpha-tocopherol, 1000 mg ascorbate, and 30

mg B-carotene) decreased markers of lipid peroxidation (MDA and breath pentane) after subjects were run to exhaustion.

Vitamin E is a fat soluble vitamin found in cellular membranes throughout the body. Researchers have examined its relationship to oxidative stress. For example, vitamin E deficient rats undergoing exhaustive exercise were found to have increased susceptibility to free radical damage and elevated markers of oxidative stress in biopsied muscles (16). This susceptibility resulted in premature exhaustion, greater fragility of lysosomal membranes and decreased muscle mitochondrial respiratory control. Vitamin E has been proposed to increase longevity by influencing the aging of the immune system (37). Immune cells contain high concentrations of vitamin E (39). Animal studies have demonstrated that vitamin E deficiency causes immune suppression (6, 38, 81) and following correction of the deficiency, immune responses return to normal levels (80). Animal studies have found enhanced immune response from vitamin E supplementation which translated into 94% increased resistance to disease and infection (57). Several studies have been performed in humans to evaluate the effectiveness of vitamin E supplementation on immune function. Meydani et al. (55) supplemented the diets of elderly individuals with 800 IU of vitamin E. After 30 days of supplementation, there was an enhancement of the DTH response and lymphocyte proliferation after mitogenic stimulation. Prasad (71) supplemented the diets of 18 young males with 300 mg vitamin E and found an enhanced bactericidal activity of peripheral blood leukocytes and cell mediated immunity.

Vitamin C is a water soluble antioxidant with a strong reducing potential. It scavenges superoxide radicals, hydroxyl radicals, peroxyl radicals, hydrogen peroxide and singlet oxygen. Like vitamin E, vitamin C (ascorbic acid, AA) has been found to reduce the effects of oxidative damage. Kaminski and Boal (46) noted in a placebo-controlled, randomized trial that 3000 mg of vitamin C for 3 days reduced muscle soreness of the posterior calf muscles. Several laboratories have studied the effect of AA on the physiology of white blood cells and preventing oxidative damage by free radicals. The concentration of AA in leukocytes is approximately 80 times greater than that found in plasma (2, 41). *In vitro* studies suggest that AA enhances human cellular immune function (17, 64). Ascorbic acid has also been shown to have positive effects on wound healing and allergic reactions. It is tolerated at levels much higher than those given in the current study even over an extended time period (36). The understanding of nutritional status, immune function and the role of vitamin C is still evolving.

Selenium is an integral component of the enzyme glutathione peroxidase which catalyzes the breakdown of hydroperoxides, and selenium deficiency in rats results in an elevation of breath pentane, a marker of oxidation (21). Although selenium alone is not an antioxidant, it plays an important role in preventing oxidative damage as a cofactor in the antioxidant enzyme system. The metabolic function of selenium with respect to the immune system has been reviewed (50, 69). Only a few human studies have been performed. For example, Peretz et al (67) supplemented the diet of institutionalized elderly subjects with 100 μg selenium/day for six months and then evaluated their immune status. The group supplemented with selenium had an

improved lymphocyte proliferative response. In another study, researchers supplemented diets with 200 μg of selenium daily for three weeks, which resulted in enhanced NK cell activity (22). Other investigators (34) have found that polymorphonuclear leukocyte phagocytic function was enhanced by the ingestion of 182.5 μg of selenium per day. These results are consistent with the animal experiments by Talcott et al (79) and Koller et al. (53). Therefore, it is generally accepted that selenium is necessary for optimal immune function and in this study it was supplied in the treatment bars at 100 μg/day. It should be pointed out that unlike the other nutrients that were supplied in the treatment bar, selenium has a narrow range for optimal health. Toxicity may occur at levels between 1500-5000 μg/day (52), and the optimal level is between the RDA (70 μg/day) and 400 μg/day.

Zinc is an important cofactor for many antioxidant enzymes, including lactate dehydrogenase, and superoxide dismutase (12, 19). Many investigators have noted that zinc deficiency affects immune functions. Pariza (65) noted that zinc deficiency caused a 25% loss of body weight in young adult mice and resulted in a 50% loss of thymic weight. They found that the antibody and cell-mediated immune responses were about half the level of normal mice. Singh et al (76) noted that blood levels of zinc and selenium were significantly reduced during US Navy SEAL training despite adequate intakes (met Military Recommended Dietary Allowances) for these minerals. Chen (12) found that zinc deficiency in mice could lead to increased free radical production that may be exacerbated by exercise which also increased urinary zinc loss.

IMMUNE FUNCTION AND STRESS

Soldiers in SFAS and RT are subjected to energy and sleep deprivation, mental stress, and intense physical exertion. Alterations in the immune system associated with stress include impairment of both the innate and specific arms of immunity. The specificity and effectiveness of the immune system is the result of various interactions among cellular elements of an immune response. Immune effector cells, such as B-lymphocytes, natural killer cells (large granular lymphocytes), and T-lymphocytes possess surface receptor and antigen recognition sites which initiate signals specific to eliciting an immune response to defend against invading pathogens or counteract internal threats from abnormal cellular transformation. B-lymphocytes are primarily involved with antibody production while T-lymphocytes are most commonly associated with cell-mediated immunity. T-lymphocytes in addition to their role as "killer cells" have important roles in regulating B-lymphocyte functions and in "orchestrating" cellular trafficking in the development of highly specific immune responses. In a clinical setting, numerous laboratory tests can be performed to evaluate immune function and have been shown to be markers of intact *in vivo* immunity (Table 1).

Table 1. Examples of laboratory immune function assessment

White Cell Numbers
        Automated cell count (complete blood count)
        Flow cytometry (differential cell population analysis by immunophenotype

analysis)

Cell Function

Lymphocyte proliferation following mitogenic stimulation
_ Tritiated thymidine uptake
_ CD69 and CD40 ligand expression
Cytotoxicity
_ NK cell cytotoxicity
_ Lymphokine activated cytotoxicity
_ Antigen specific CD8+ cytotoxicity
Cytokine production
T- and B-lymphocyte cooperation
_ CD40 ligand expression
Immunoglobulin production
_ Antibody response to vaccination

In Vivo Immunity

Cutaneous reaction
_ Delayed-type skin hypersensitivity
Resistance to viral infections
_ Rhinovirus challenge
Incidence of infectious disease

In vitro tests may not in all cases be indicative of clinically relevant immune deficiencies. Furthermore, *ex vivo* studies using blood cells need to be performed on fresh samples and a single test will usually not be predictive of overall immune system status. Therefore, when evaluating immune status or competency, it is important to assess a variety of functions or components. It is also important to attempt to quantitate overall immune effectiveness by using clinical measures such as DTH, rate of infection or response to pathogen or vaccine. A study by Christou et al. (13) has shown good correlation between lack of DTH reactivity (anergy) and risk of infection as well as mortality in a hospital setting (Table 2). Patients that are DTH reactive have a much lower rate of infection while those patients who are anergic have not only a greater risk of infection, but also a less favorable prognosis. Reduced cell mediated immunity can limit the development of an effective immune response against pathogens, including viruses, bacteria, fungi, and protozoa. Previously, we have found that training stress decreases lymphocyte activation and alters circulating T- and B-lymphocyte subsets. We found that 30-50% and 50-70% of the soldiers participating in SFAS and RT, respectively, have suboptimal DTH responses (48).

Table 2.   Infection and death rates based on delayed-type hypersensitivity skin testing of 4,289 hospitalized patients (13)

	Infection Rates	Death Rates of Infected
Reactive[#]	172/2509 (7%)	33/172 (19%)
Relative Anergy*	109/666 (16%)	35/109 (32%)
Anergy**	289/1114 (25%)	147/289 (51%)

[#] Intradermal injection into the forearm of *Candida*, Mumps skin test antigen, purified protein derivative, trichophyton and varidase. Reactive was defined as a diameter of induration equal to or greater than 5 mm..
    *Response to one of five antigens
    **No response to any of the antigens

The purpose of this study was to quantify the antibody response to hepatitis A vaccine during the stress of RT and determine the immunological influence of a unique nutritional bar on vaccine response. Humoral immunity to protein antigens or conjugate vaccines requires the cooperation of T-lymphocytes. Antigens which are "thymus dependent" require T helper lymphocytes to initiate the signals for B-lymphocyte differentiation and proliferation, immunoglobulin (Ig) isotype switching and antibody production. The B-lymphocytes' response to antigen stimulation can be primary (naive) or secondary (memory). The primary antibody response is the result of a coordinated reaction to antigen involving antigen presenting cells and T- and B-lymphocytes. Hepatitis A vaccine in this study (Havrix[R], SmithKline Beecham) requires a T-cell dependent mechanism to elicit a specific anti-hepatitis A antibody response (43). We vaccinated soldiers with hepatitis A (Havrix[R]) vaccine during the stress of RT. Blood was obtained at pre- and post-vaccination to determine the impact of RT stress (chronic stress) as well as nutrient supplementation in a food form on the neuroendocrine and immunologic response.
    The objectives of this study were:
1) To quantify the antibody response to a vaccine (Havrix[R]) during the stress of RT.
2) To compare the immunologic effect (antibody response to vaccine-Havrix[R]) of an isonitrogenous and isocaloric placebo bar to a nutritional bar containing high levels of antioxidants, vitamins, structured lipid (canola and MCT oils), carnitine, taurine and indigestible carbohydrates during the stress of RT. Furthermore, compare the immunologic responses in stressed soldiers with a cohort of age-matched, non-stressed soldiers.
3) To compare the immunologic impact (lymphocyte phenotype, activation by phytohemagglutinin (PHA) or phorbol-12-myristate-13-acetate (PMA), function, and delayed-type hypersensitivity) and nutritional status of trainees consuming nutritional bars.
4) To evaluate the acceptance of a novel nutritional bar with respect to flavor, organoleptics and perceived benefit during RT (subject questionnaire).
5) To compare the flavor of this bar to other nutritional bars consumed by soldiers.
6) To develop new biomarkers of immune function to further define the relationship between stress and infectious disease

MATERIALS AND METHODS

STUDY DESIGN

The Ranger Training Class commencing on October 9, 1997 at Ft Benning, GA, was utilized to screen volunteers for this study. Individuals were briefed on the purpose, risks, and potential benefits of participation in the study. The briefing of the volunteers was conducted independently and in the absence of any members of the immediate chain of command, to prevent any conflict of interest or undo pressure on the volunteers. Individuals who did not wish to participate in the assessment part of the study (i.e. blood draws and vaccination) were allowed to be randomized to receive the supplement provided they consented to monitoring and participation in surveys. The volunteers were informed that they could withdraw from the study at any time without penalty, and that their participation would not influence their status during RT. Once volunteers were enrolled into the study, they were randomly assigned to receive the placebo or treatment bar (the non-stressed reference group received the vaccine but did not consume a bar). The nutritional bars (65 grams) were isonitrogenous and isocaloric, developed and supplied by Ross Products Division of Abbott Laboratories. The bars consisted of protein, fat, carbohydrate (285 kcal, total), vitamins, minerals, trace elements, taurine, and carnitine. (Precise formulation is the proprietary information of the Ross Products Divison of Abbott Laboratories). Control bars were isocaloric and isonitrogenous, but lacked key immune-enhancing ingredients.

One hundred ninety soldiers volunteered to participate in all aspects of this study. Subjects were blinded to the treatment and randomly assigned to consume bars with blue, red or yellow stickers. The three color codes were established to discourage the subjects from attempting to break the code. Subjects were given one bar in the morning and one bar in the evening starting on October 19, 1997, and continuing through December 2, 1997. Consumption was monitored by direct observation during the study by training course instructors. On occasion, two bars were provided in the morning to each subject when it was not feasible to deliver bars in the evening. There was also a group (n=9) of age-matched soldiers who were not undergoing the same stress but were used as a non-stressed reference group. This group of soldiers did not consume any bars but did receive a vaccination and had their blood drawn. Blood was drawn from every subject between 2200 and 0100 hours and ninety-six individual samples were randomly assigned to have flow cytometry analysis performed. Subjects whose blood samples were initially analyzed by flow cytometry were followed throughout the study; however, study subjects dropping out of RT were replaced by others consuming the same bars from the beginning of the study (to gain as much immunological information as possible).

Blood samples were drawn at baseline-visit one (Fort Benning, GA); during the most stressful training or the mid-mountain phase before vaccination-visit two (Dahlonega, GA); end of the mountain phase-visit three (Day 14 after vaccination); end of RT, day 28 after vaccination-visit four (Eglin AFB, FL). Table 3 describes the type of blood collection, analysis performed and rationale for specific tests. At baseline, the soldiers' blood samples were screened for antibodies

against hepatitis A using the clinical laboratory protocol for the Abbott Laboratories' IMX system (North Chicago, IL). Soldiers that tested positive by the IMX system were not vaccinated.

Table 3. Blood collection tube, analysis, and rationale for the specific tests.

Tube	Analysis	Rationale
Red Top (Serum Tube)	Antibody	Determine vaccine response and the effect of stress on biomarkers of immune function. Further determine whether nutritional modifiction altered antibody response to a vaccine challenge.
Purple Top (EDTA Tube)	Whole blood stimulation and cytokine concentration	Determine cytokine expression in circulation as well as compare cellular responses to previous studies
Blue Top Sodium (Heparin Tube)	Flow cytometry analysis	Determine lymphocyte phenotype populations, lymphocyte activation (CD69 expression), B-lymphocyte cooperation, and intracellular cytokines

Figure 1 indicates the time-line of the study. Ranger Training comprises 3 phases: Benning Mountain, and Swamp. The mountain phase is the most arduous and stressful. During the mid-mountain phase blood samples were collected and volunteers were vaccinated with Havrix[R] (SmithKline Beecham). Subsequent blood samples were taken 14 and 30 days from the time of vaccination. DTH skin tests were given immediately after the first and last blood draw and read 24 and 48 hours later (Figure 1). Multitest-CMI was administered to 30 subjects at baseline to determine average responsiveness and to the remainder of the subjects at the end of the study to determine post stress responsiveness as well as determine if there was a difference between the two groups consuming the bars.
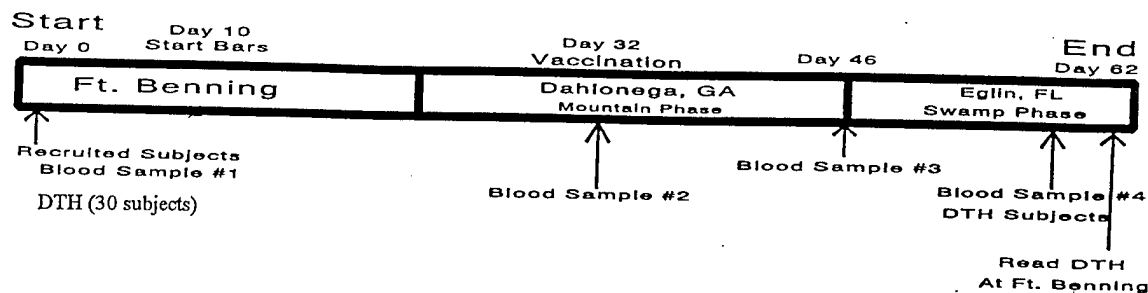
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Start        Day 10                    Day 32                              End
Day 0        Start Bars               Vaccination        Day 46            Day 62
┌──────────────────────┬──────────────────────────────┬──────────────────────────┐
│      Ft. Benning     │      Dahlonega, GA           │       Eglin, FL          │
│                      │      Mountain Phase          │      Swamp Phase         │
└──────────────────────┴──────────────────────────────┴──────────────────────────┘
  Recruited Subjects                          Blood Sample #3
  Blood Sample #1
  DTH (30 subjects)              Blood Sample #2              Blood Sample #4
                                                              DTH Subjects

                                                              Read DTH
                                                              At Ft. Benning
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Figure 1. Study Design

VACCINE

Soldiers received a vaccination against hepatitis A (Havrix[R]) during the most severe stress of RT. This vaccine was used because of its safety profile (low risk of adverse reactions) and the fact that soldiers would be receiving it at some point in their Army career. The vaccine is an inactivated, non-infectious hepatitis vaccine developed and manufactured by Smith-Kline Beecham Biologicals. The virus (strain HM175) is grown in MRC human cells, isolated, and then inactivated using formalin. Blood samples were obtained pre- and post-vaccination to determine the impact of RT stress (chronic stress) as well as nutrient supplementation in a food form, on neuroendocrine and immunologic responses.

NUTRITION BARS

Bars were formulated and manufactured by Ross Products Division of Abbott Laboratories. Formulations for each product contained similar amounts of energy yet differed in micronutrient composition. The placebo and treatment nutritional bars (65 grams) were isonitrogenous and isocaloric. In previous studies, nutrient intake from the soldiers' normal rations met or exceeded the military dietary allowances (MRDA) for all nutrients except folic acid (SFAS=276 mg; RT 284 mg, ~70% MRDA) and vitamin E (SFAS 7.4 mg; RT=6.8,~70% MRDA). Other nutrients which were close to 100% of the MRDA included zinc and iron. Therefore, nutrients included in the treatment bar covered any dietary nutrient deficits and supplied increased levels of nutrients that potentially may be immunologically beneficial under stressful conditions (see nutrients section of introduction).

Soldiers received nutritional bars during RT as a means to determine if this novel nutritional formulation benefited the soldier immunologically during intense stress. We do not believe that clinically relevant vitamin and mineral deficiencies developed or were manifested during RT;

however, the intense training may cause increased nutrient requirements of various nutrients above the MRDA. In addition, maintaining nutrient levels may benefit the soldiers by reducing illness, injury or risk of infectious diseases during stress. We have chosen modest levels of nutrients that have the potential to affect the immune response without jeopardizing safety. Soldiers were not allowed to consume any other nutritional supplements during RT.

ANTIBODY QUANTITATION

Subjects were vaccinated intramuscularly with Havrix[R] according to specifications from the manufacturer (SmithKline Beecham Biologicals). Army medical personnel administered all vaccinations at the RT site in Dahlonega, GA during the middle of the mountain phase of RT (peak stress). The study medical monitor was present at all vaccinations and blood collections. Hepatitis exposure or recent vaccination was evaluated by personnel at Martin Army Community Hospital, Ft. Benning, GA, using the IMX[R] system (Abbott Laboratories, North Chicago), a qualitative method to establish recent exposure or vaccination. The subjects with positive antibody tests or confirmed history of hepatitis were not vaccinated. Quantitative anti-hepatitis antibody analysis was performed by Abbott AxSYM[R] HAVAB 2.0 Quantitative. This is a microparticle enzyme immunoassay (MEIA) designed for the quantitative detection of total (IgG;IgM) antibody to Hepatitis A virus (HAV). The assay is intended for the diagnosis of previous or ongoing HAV infection as well as to help establish patient immunity. Anti-HAV is detectable during the acute phase of Hepatitis A virus infection (anti-HAV IgM) and may persist for years after recovery (anti-HAV IgG). The primary clinical utility for the assay is to determine previous exposure to HAV. Additional uses include the indication of past or present HAV infection, to assess patient's immune status, and to monitor vaccine response. The AxSYM[R] HAVAB 2.0 Quantitative is currently under development. We used this assay to establish and quantitate antibody responses to the immunization. The dynamic range of the AxSYM[R] HAVAB 2.0 Quantitative assay is 0-100 mIU/mL. Samples with antibody concentrations >100 mIU/mL were diluted only in the subjects that were evaluable. Medical records were monitored by medical personnel to track illness, injury and medication use (data not shown).

FLOW CYTOMETRY

Blood samples (7 ml) were collected via venipuncture with sodium heparin as the anticoagulant. Vacutainers containing the blood sample were labeled with coded study numbers to maintain anonymity and placed in test tube racks, sealed in watertight plastic bags, then placed into an insulated carrier for shipment. Samples were delivered to the laboratory within 6 to 10 hours after collection. On receipt, blood samples were immediately assigned a Ross sample number and prenumbered labels were attached to each tube. A record linking the study number with a Ross number was completed. All whole blood samples were processed at room temperature. Standard biohazard class 2 and barrier precautions were observed at all times during the laboratory procedures.

Flow cytometric determination of a five-part differential white cell count in blood samples

were performed; the monoclonal antibody panels and the key cell populations are enumerated in Table 4.

Table 4. Monoclonal antibody panel and key cellular populations identified.

| Tube | Antibody labeled with specific fluorochome | | | | Cell Types Detected | Key Cell Populations Measured |
	FITC	PE	PerCP	APC		
1	IgG1	IgG1	IgG1	IgG1	Isotype controls	
2	IgG1	IgG1	IgG1	IgG1	Isotype controls	
3	CD16	CD14	CD45	CD3	5-part differential count, monocyte phenotype gating	Lymphocytes, monocytes, neutrophils, eosinophils, basophils CD14+/CD16-, resting monocytes CD14+/CD16+, activated, differentiating monocytes
4	Vγ9	Vδ2	CD3	CD4	γδ T-lymphocyte subtypes	Vγ9+Vδ2+CD3+ CD4-, predominant γδ T cells Vγ9-Vδ2-CD3+CD4-, DTH disease phenotype, CD8 cells
5	CD45RA	45RO	CD8	CD4	Naive/Memory helper or cytotoxic T-lymphocytes	CD45 RA+/45RO-/CD4+/CD8-, Naive helper T cells CD45RA-45RO+/CD4+/CD8-, Memory helper T cells CD45RA+/45RO-/CD4-/CD8+, Naive cytotoxic T Cells CD45RA-/45RO+/CD4-/CD8+, Memory Cytotoxic T Cells
6	TCRγδ	CD16 &56	CD3	CD95	T-lymphocytes, NK cells, γδ T-lymphocytes, T-lymphocytes FAS antigen status	γδ+/16&56-/CD3+/CD95-,γδ Tcells, non-apoptotic γδ-/16&56-/CD3+/CD95-,αβ T cells, non apoptotic γδ+/16&56-/CD3+/CD95+,γδ T cells, apoptosis primed γδ+/16&56+/CD3/CD95+,αβ NK cells, apoptosis primed
7	CD69	CD23	CD19 &20	CD38	B-lymphocytes, B-lymphocyte activation	CD69+/CD23-or+/CD19&20+/CD38+, endogenously activated B cells CD69+/CD23+or-/CD19&20/CD38+, endogenously activated T or NK cells

Mitogen stimulation (phytohemagluttinin, PHA) of B- and T-lymphocyte (CD69 expression) subsets was analyzed using the following monoclonal antibody panel.

| Antibody labeled with specific fluorochome | | | | Cell Types Detected | Key Cell Populations Measured |
FITC	PE	PerCP	APC		
γδ	CD69	CD3	CD19	Non- and mitogen treated (4 hr PHA stimulated) T-, B-lymphocyte activation	γδ+/CD69+/CD3+/CD19-, intrinsically activated and stimulated γδ T-lymphocytes γδ-/CD69+/CD3+/CD19+, intrinsically activated and stimulated αβ T-lymphocytes γδ-/CD69+/CD3-/CD19+, iintrinsically activated and stimulated B-lymphocytes, γδ-/CD69+/CD3-/CD19&20- intrinsically activated and stimulated NK cells.

Flow cytometric measurement of mitogen-stimulated (phorbol-12-myristate-13-acetate, PMA + ionomycin) activation of CD40L and CD69 in mature and immature lymphocytes was made using the following monoclonal antibody panel:

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Antibody labeled with specific
fluorochome

FITC	PE	PerCP	APC	Cell Types Detected	Key Cell Populations Measured
CD40L	CD69	CD3	CD45RO	Non-mitogen-treated and mitogen treated (4 hr PMA+ionomycin stimulated) B-, T-lymphocyte activation, maturation, and interactive capability	CD40L+/CD69+/CD3+/CD45RO+,intrinsically activated and stimulated mature T-lymphocytes CD40L+/CD69+/CD3+/CD45RO-, intrinsically activated and stimulated immature T-lymphocytes CD40L-/CD69+/CD3-/CD45RO+, intrinsically activated and stimulated mature NK and B-lymphocytes

Antibody labeled with specific
fluorochome

FITC	PE	PerCP	APC	Cell Types Detected	Key Cell Populations Measured
IFNγ	IL-4	CD4	CD3	Non-mitogen treated and stimulated (4 hr PMA+ionomycin) Th1, Th2, Tc1, Tc2 lymphocytes	IFNγ+/IL4-/CD4+/CD3+, Th1 Lymphocytes IFNγ-/IL4+/CD4+/CD3+, Th2 Lymphocytes IFNγ+/IL4+/CD4+/CD3+, Th0 Lymphocytes IFNγ+/IL4-/CD4-/CD3+, Tc1 Lymphocytes IFNγ-/IL4+/CD4-/CD3+, Tc2 Lymphocytes IFNγ+/IL4+/CD4-/CD3+, Th0 Lymphocytes
IgG1	IgG1	IgG1	IgG1	4 hr. PMA+ionomycin stimulation isotype controls for anti-cytokine stained cells	

These panels were used to evaluate specific cellular populations and determine what cellular changes occurred in stressed subjects and whether the treatment bar minimized or prevented these stress induced changes when compared to the placebo group.

DELAYED TYPE HYPERSENSITIVITY (DTH) TEST

In vivo immune responses were assessed by administering a DTH test, Multitest-CMI (Connaught Laboratories, Inc., Swiftwater, PA). A lack of response (anergy) can indicate either no prior exposure to the antigens contained in the test or a state of compromised cellular immune function. Furthermore, a lack of response has been correlated with increased morbidity and mortality in a variety of conditions and disease states. "Cutaneous anergy may indicate functional impairment of, or abnormalities in, the cellular immune system. Delayed cellular hypersensitivity is a valuable measure of immune response because it involves a complex series of immunologic, cellular, mediator-associated, and vascular effects (Connaught Laboratories, Product information, page 2)." This type of test has been utilized for many years as a measure of immune responsiveness in patients experiencing malnutrition (24, 44, 54, 62). Multitest-CMI was administered to 30 subjects at baseline to determine average pre-stress responsiveness. The

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remainder of the subjects were tested post-stress to assess their responsiveness as well as determine if there was a difference between the two groups consuming bars. The test kits contain a glycerin negative control and seven antigens prepared from culture filtrates of the following microorganisms: *Clostridium tetani* (tetanus toxoid antigen), *Corynebacterium diphtheriae* (Diphtheria toxoid antigen), *Streptococcus* (Group C), *Mycobacterium tuberculosis* (C, D, and PN) plus *Mycobacterium bovis* (Vallee) *Candida albicans* (Candida antigen), *Trichophyton mentagrophytes* (trichophyton antigen), *Proteus mirabilis* (Proteus Antigen). A small amount of soluble antigen is introduced into the epidermis with the applicator. The intensity of the response peaks between 24 and 72 hours after application. The test kit contains antigens that the study participants should have been exposed to (particularly tetanus) and therefore anergy would indicate some level of immune dysfunction. All of the tests were administered by one individual to reduce the variability of administration. Antigens were placed on the left forearm after the area had been cleansed with alcohol. After 24 and 48 hours the tests were read to determine the size of induration. Erythema without induration was not considered significant. An induration greater than 2 mm was considered a positive response. Whenever a positive skin test was noted, measurements were made perpendicular across the induration and the largest response (mm) was recorded. Furthermore, each response was measured and a total response was calculated by summing the total mm response. Previous studies have determined that approximately 1.2% (n=315) of normal healthy male subjects are anergic while the average total mean score is 18.3 mm with a 4.5 /individual average number of positive responses (51).

QUESTIONNAIRES

Each participant completed surveys regarding their health and psychological assessment as well as a questionnaire of bar flavors and preference. The latter contained questions regarding overall liking, flavor, coating, saltiness, sweetness, and texture. Subjects also were asked whether they thought the bar was of help in completing RT, and their preference regarding bar size. Lastly, the subjects were asked whether they had eaten other nutrition or sports bars and to compare their preference to the study bar. All surveys were kept confidential and maintained by the principal investigator.

STATISTICS

The following is a summary of the statistical methods used in this study. Blood samples were obtained at four time points during Ranger Training: 1) Fort Benning, 2) mid-mountain phase, 3) end of mountain phase, and 4) end of swamp phase (end of study). For most of the data, visit one was the baseline and therefore used to evaluate changes over time (there were three calculated changes): visit two minus visit one, visit three minus visit one, and visit four minus visit one. For the antibody response to hepatitis A vaccination, visit two served as a baseline since this was performed immediately prior to vaccination. The primary outcome in this study was antibody response to the vaccine at two and four weeks post vaccination. Antibody responses were compared at baseline (visit two), 14 (visit three-visit two) and 30 days (visit four-visit two) post vaccination. Subjects were also classified whether or not a protective antibody level > 20

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mIU/mL (60, 92) had been reached (seroconversion). The two groups were compared between distribution of subjects in the two categories (Yes/No) with a Two-sided Fisher's Exact Test at visit two, visit three, and visit four. A type-I error rate of 0.05 was considered statistically significant.

The DTH data was ordinal in nature. The responses were measured to eight different antigens and added together for a total score. Subjects tested at visit one and visit four were different subjects and therefore were not assessed individually as to a change over time. The responses for the baseline and completion groups' tests were examined with a Two-sided Fisher's Exact Test at each time point. A two Sample t-Test on ranked data was used at each time point to analyze the calculated total score.

Flow cytometry determined the percentage of a specific cell types, and cell numbers were calculated based on white blood cell counts. Cell numbers were calculated as the number of cells per μL of blood. For most of the data, groups were compared based on data obtained at baseline (visit one) and the three calculated changes from baseline. A two sample T-test was used to compare the groups. The residuals obtained from fitting the model were examined with the Shapiro-Wilk test for assessing if the residuals were normally distributed. Any parameters for which there was evidence that the residuals were not normally distributed ($p<0.05$) at one or more time points were analyzed with nonparametric methods. This consisted of ranking the data and then analyzing the ranks with the Two Sample t-Test, in essence the Wilcoxon Rank Sum Test. If significant differences were observed at visit one between the two groups, an Analysis of Covariance (ANCOVA) was used to analyze the values of visit two to visit four, the residuals were examined for meeting the assumption of being normally distributed. If the hypothesis of normality was rejected (using $P<0.05$ for the Shapiro-Wilk test) at any step of the process, the analyses were all repeated utilizing ranked data for the Analysis of Variance of visit one values and normal scores for the ANCOVA for visit two to visit four values.

The calculated changes within each group were examined in an attempt to determine the influence of stressful training over time. The calculated changes were examined with a One-Sample t-Test which was a test of the hypothesis that the calculated change was zero (ie. no change). In addition, the data was examined with the Shapiro-Wilk test for the assumption of being normally distributed. If the assumption was rejected ($P<0.05$ for the Shapiro-Wilk Test) for one or more of the calculated changes for the particular parameter, then the One Sample t-Test was replaced with the nonparametric equivalent, the Wilcoxon Sign Rank Test, which tested the hypothesis that the median (rather than the mean) is zero. The majority of the parameters exhibited a non-normal distribution at one or more of the time points under consideration and therefore, for consistency with the earlier analyses on two groups, all analyses were conducted on

ranked data. Caution was used when interpreting the analyses of the individual groups because the treatment effects could only be determined from the two group analyses.

No adjustments were made to the significance levels for multiplicity of testing. All results were considered statistically significant if the significance level was =/< 5%. Some trends were indicated (e.g. body weight) with a significance level above 0.05 and below 0.10.

RESULTS

Of the 190 subjects that volunteered for the study, only 136 were available to be randomized to the placebo or treatment groups. This decrease in study volunteers represents attrition during the first 7 days of the course, prior to the start of the nutritional supplement. There were a total of 90 subjects that completed the course and the study attrition rate was 37%. The total number of subjects vaccinated and those who completed RT based on treatment group are noted below, Table 5. Analysis by the qualitative method (Abbott AxSYM^R HAVAB 2.0) indicated that there were 4 subjects in each group who had background levels above 5mIU/mL indicating exposure to hepatitis A virus but not identified on the IMX^R. These subjects experienced a secondary response and were not included in the overall response curves. There was also an aged-matched group (n=9) of soldiers who were not undergoing strenuous training stress but were used as a non-stressed reference group. This group of soldiers did not consume any bars but did receive a vaccination and had their blood drawn for analysis.

Table 5. Number of subjects who participated, randomized and vaccinated during RT

	Placebo Group	Treatment Group
Subjects originally randomized and consumed bars	60	63
Subjects completing Ranger Training	43	47
Subjects vaccinated	17	26
Evaluable subjects who were vaccinated and completed Ranger Training	13*	21*

*There were 4 subjects in each of the placebo and treatment groups that had background levels of antibodies (>5mIU/mL) and were therefore excluded from the analysis. One subject in the treatment group did not show up for the final blood draw.

ANTIBODY RESPONSE

The mean average antibody titers of subjects between groups at 14 and 28 days post vaccine are represented in Figure 2. There was no difference between the two treatment groups; however, the stress imposed upon the soldiers decreased the antibody response (-29% to -36%, placebo and treatment respectively) as compared to the non-stressed reference group. All of the subjects (7/7, 100%) in the non-stressed reference group obtained a protective antibody titer (>20

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mIU/mL) by day 28 whereas 2/17 (11.8%) and 5/23 (21.7%) did not for the placebo and treatment group respectively (NS, $P=0.68$).
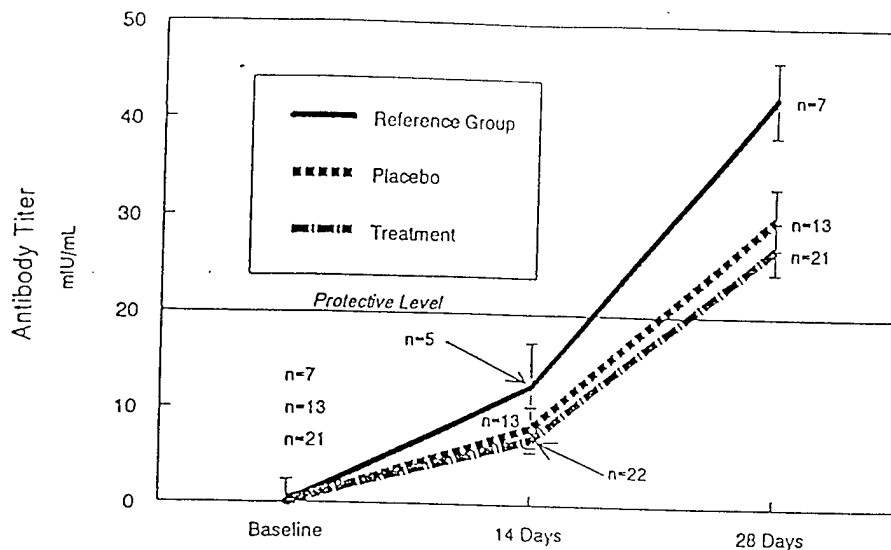

Figure 2. Average antibody responses (primary) to hepatitis A vaccine (Havrix[R]). Soldiers consumed either treatment or placebo bars (mean +/- SEM). Also compared to a non-stressed military reference group.

BODY WEIGHT

The soldiers in the treatment and placebo groups were similar in height (68.7+/-0.4 inches and 68.7+/-0.3 inches) and body weight (165.8+/-2.4 pounds and 168.2+/-2.2 pounds) at the beginning of the study. There was little change in body weight during the Fort Benning phase of the training; however, during the mountain phase both groups experienced a similar pattern of body weight loss of -5.5+/-0.7 pounds (range of -14.9 to +6.10) and -6.0+/-0.6 (-15.9 to +7.5) pounds for the treatment and placebo groups, respectively. This pattern of weight loss is substantially different from previous Ranger studies which typically demonstrated an average body weight loss of 20 to 30 pounds over the duration of the course. Both groups experienced an average weight gain over the course and a trend ($P=0.068$, Two Sample t-Test) toward larger weight gain in the treatment group.
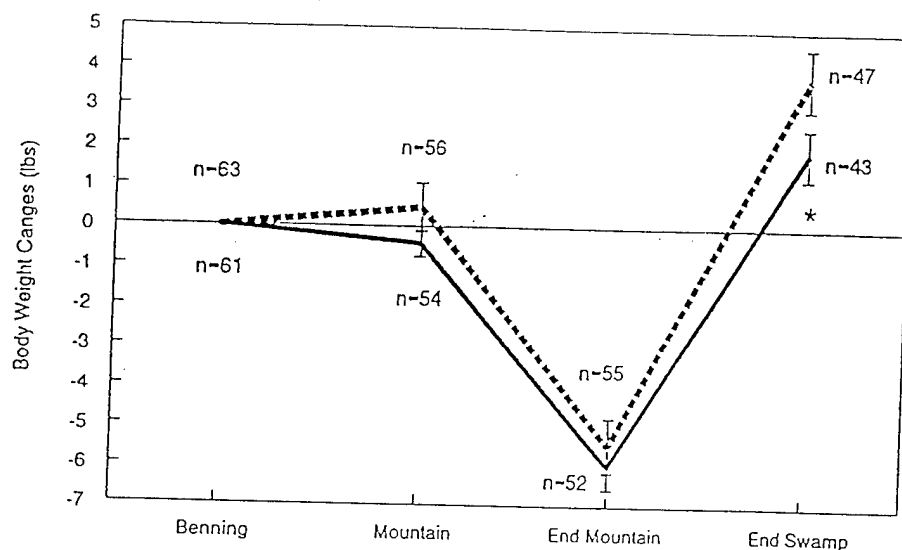
Figure 3. Body weight changes (mean +/- SEM) of soldiers during Ranger training. The treatment group is represented by the dashed line and the placebo by the solid line, *P=0.068.

DELAYED TYPE SKIN HYPERSENSITIVITY

Thirty subjects (n=16 Placebo and n=14 Treatment groups) were tested at baseline to establish their immune status at baseline, while 55 subjects (n=26 Placebo and n=29 Treatment) received the skin test upon completion of the course, to determine if there was a difference between the treatment and placebo groups. The subjects tested at baseline had a greater (23-38% total mm response when compared to those who completed RT; however, since the subjects were not the same, we did not compare them statistically. The physical rigors of RT caused a less vigorous DTH response and the treatment did not attenuate this decrease (see Table 6) as it had done in Specials Forces School (48).

Table 6. Total mean DTH induration of Multitest CMI of soldiers consuming placebo or treatment bars during RT, SEM in parentheses.

Groups	Baseline Induration	Anergic Soldiers at Baseline	End Induration	Anergic Soldiers at End
Placebo	8.44 (1.1)	12.5%	6.54 (1.02)	37%
Treatment	8.29 (.97)	14.3%	5.10 (.95)	41.9%
Non-stressed	13.6 (1.8)	0.0%		

FLOW CYTOMETRY

White Blood Cells

There were no statistical differences in the white blood cell (WBC) counts between the two groups at any time point. However, within each group over time (visit 2-1, visit 3-1, visit 4-1), there was a marked decrease in the number of WBC ($x10^3$ cells/μL). Furthermore, there was a decline in total leukocytes and most particularly, in lymphocyte cell number (Table 7). There was a statistically significant greater decline of monocytes from baseline to the second ($P=0.041$) and third ($P=0.013$) time points in the placebo group as compared to the treatment group. There were no differences between the number or percentage of neutrophils between the two groups; however, it appears that the subjects had more neutrophils than the reference group at baseline and continued to be higher throughout the study, possibly indicating that the soldiers were stressed before starting RT.

Table 7. White blood cell counts, lymphocytes and neutrophils populations of soldiers participating in Ranger Training and consuming placebo or treatment bars. The number with the superscript number in parenthesis indicates the number of subjects in the group (n) while the non-italicized values in parenthesis represents the mean change from baseline.

	Visit 1	Visit 2	Visit 3	Visit 4
White Blood Cell Count (x10³ cells/μL)				
Placebo	$10.17^{(39)\#}$	$7.29^{(45)}(-2.97\#)^{(35)}$	$8.16^{(43)}(-2.25\#)^{(34)}$	$7.85^{(43)}(-2.27\#)^{(29)}$
Treatment	$9.63^{(47)}$	$7.21^{(51)}(-2.37\#)^{(42)}$	$8.22^{(49)}(-1.35\#)^{(41)}$	$7.66^{(49)}(-2.04\#)^{(37)}$

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Lymphocytes (Percent)				
Placebo	$32.7^{(39)}$	$37.6^{(44)} (4.7\#)^{(35)}$	$32.8^{(43)} (0.25)^{(34)}$	$29.3^{(44)} (-3.3)^{(29)}$
Treatment	$31^{(47)}$	$34.9^{(51)} (4.0\#)^{(42)}$	$31.6^{(49)} (0.6)^{(41)}$	$29.6^{(50)} (-0.3)^{(37)}$
Reference Group		$38.2^{(9)}$	$34^{(5)} (-2.8)^{(5)}$	$33.5^{(8)} (-4.4)^{(8)}$
Lymphocytes (x10³ cells/μL)				
Placebo	$2.53^{(60)}$	$2.29^{(56)}(-0.33\#)^{(52)}$	$2.4^{(54)} (-0.22\#)^{(51)}$	$2.1^{(44)} (-0.44\#)^{(42)}$
Treatment	$2.43^{(62)}$	$2.22^{(65)}(-0.28\#)^{(54)}$	$2.3^{(65)} (-0.16\#)^{(54)}$	$2.1^{(54)} (-0.29\#)^{(47)}$
Neutrophils (Percent)				
Placebo	$55.2^{(39)}$	$50.6^{(44)} (-4.5\#)^{(35)}$	$55.3^{(43)} (-0.17)^{(34)}$	$59.0^{(45)} (4.8\#)^{(29)}$
Treatment	$57.3^{(47)}$	$52.6^{(51)} (-5.0\#)^{(42)}$	$56.4^{(49)} (-0.70)^{(41)}$	$59.5^{(50)} (1.3)^{(37)}$
Reference Group		$51.8^{(9)}$	$55.6^{(5)} (3.5)^{(5)}$	$52.1^{(8)} (0.44)^{(8)}$
Neutrophils (x10³ cells/μL)				
Placebo	$5.8^{(39)}$	$3.7^{(44)} (-2.1\#)^{(35)}$	$4.5^{(43)}(-1.4\#)^{(34)}$	$4.7^{(43)}(-1.0)^{(29)}$
Treatment	$5.6^{(47)}$	$3.8^{(51)} (-1.8\#)^{(42)}$	$4.7^{(49)} (-0.9\#)^{(41)}$	$4.6^{(49)} (-1.1\#)^{(37)}$
Reference Group		$3.6^{(9)}$	$3.5^{(5)} (0.3)^{(5)}$	$3.9^{(8)} (0.2)^{(8)}$
Monocytes (Percent)				
Placebo	$5.0^{(39)}$	$6.2^{(44)} (1.3\#)^{(35)}$	$4.8^{(43)} (-0.11)^{(34)}$	$6.1^{(45)} (0.75\#)^{(29)}$
Treatment	$5.2^{(47)}$	$6.6^{(51)} (1.4\#)^{(42)}$	$5.0^{(49)} (-0.32)^{(41)}$	$6.0^{(50)} (0.68\#)^{(37)}$
Reference Group		$6.2^{(9)}$	$6.0^{(5)} (-.84)^{(5)}$	$7.3^{(8)} (0.79)^{(8)}$
Monocytes (x10³cells/μL)				
Placebo	$0.99^{(60)}$	$0.87^{(56)}(-0.15\#)^{(52)}$	$0.80^{(54)} (-0.21\#)^{(51)}$	$0.74^{(44)} (-0.26\#)^{(42)}$
Treatment	$0.92^{(62)}$	$0.86^{(65)} (-0.06*)^{(54)}$	$0.83^{(65)}(-0.10*\#)^{(54)}$	$0.76^{(54)} (-0.19\#)^{(47)}$

#number of subjects (n) at each time point and used to calculate differences
\# P<0.05 within the group
* P<0.05 among the groups

Lymphocytes

There were several lymphocyte changes that occurred over time (within each group) as the result of the soldiers' training stress. The major changes between placebo and treatment groups that occurred which have clinical implications are described below with specific differences identified.

Overall, in the comparison between groups, there was a smaller decline in the treatment group's absolute T-lymphocyte numbers at time point 4 (end of the study) but little change in the

percentage of T-lymphocytes (see Figure 4). The sub-populations of helper/inducer T-lymphocytes showed a similar pattern (likely contributing to the reason for the drop in total T-lymphocytes). The placebo group had a larger decrease in the number of helper/inducer T-lymphocytes (see Figure 5). A similar pattern was observed for the cytotoxic/suppressor (CD4-) T-lymphocytes ($P=0.042$) and memory helper/inducer (CD4+/RA-/RO+) T-lymphocytes ($P=0.019$) at the end of the study. Trends were also similar ($P=0.058$) at the end of the mountain phase (visit 3) for the cytotoxic/suppressor naive (CD8+/CD4-/RA+/RO-) T-Lymphocytes.

The number of subjects at each time point used to calculate cell type means and those used to calculate change in percentage or change in cell numbers for the flow cytometry analysis are shown in Table 8.

Table 8. (A) Number of subjects used to calculate time point means, and (B) to calculate change in percent and lymphocyte numbers. Subject numbers for calculation of changes are lower because samples were not always available for baseline or subsequent time points.

A.

	Visit 1	Visit 2	Visit 3	Visit 4
Placebo	n=39	n=44	n=43	n=45
Treatment	n=47	n=51	n=49	n=50

B.

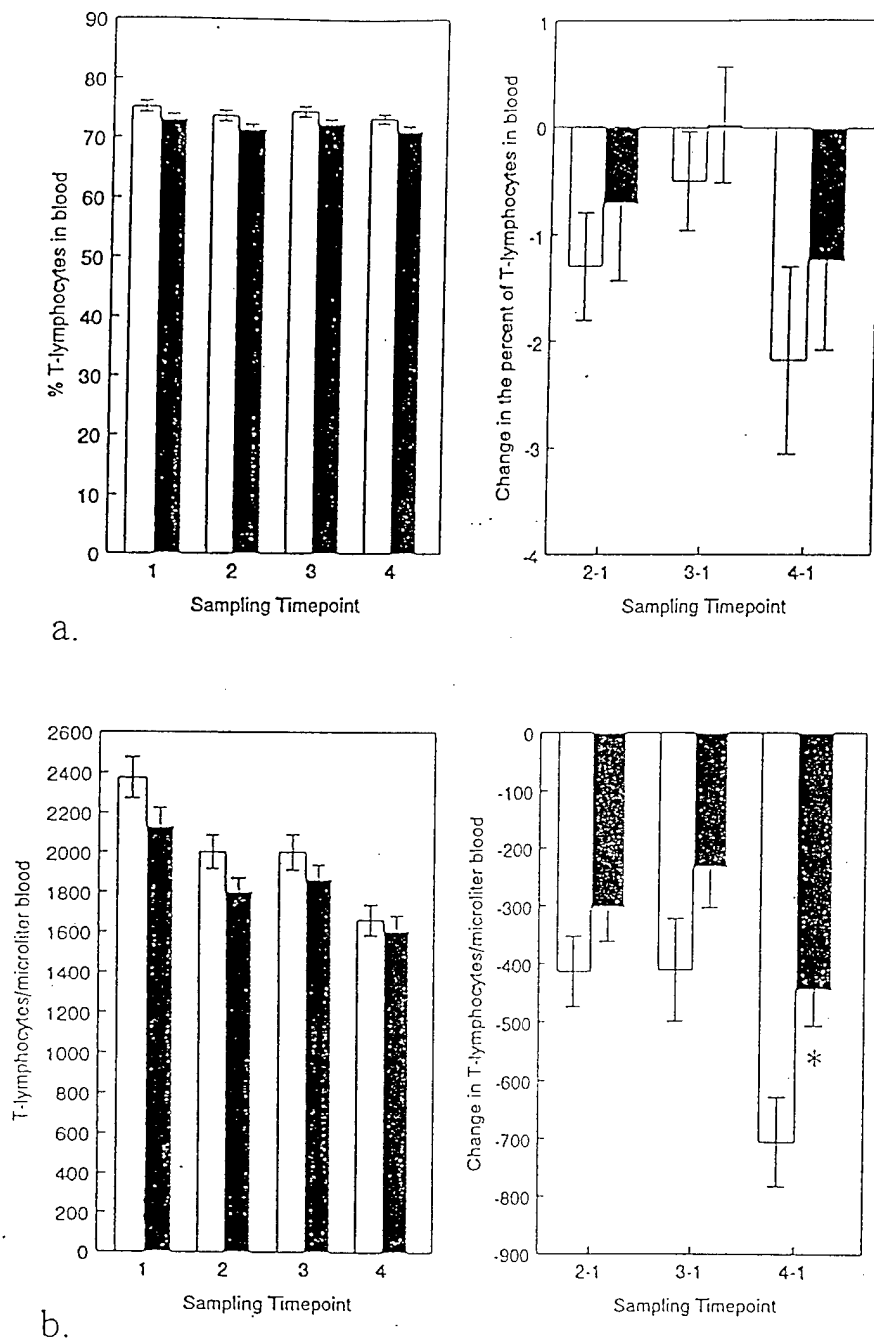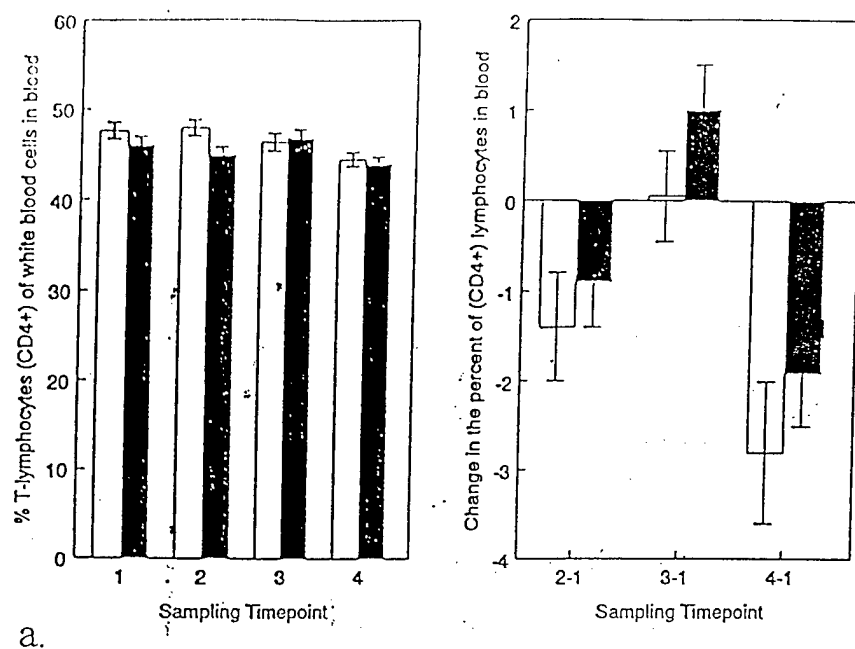|	Visit 2-1	Visit 3-1	Visit 4-1
Placebo	n=35	n=34	n=29
Treatment	n=42	n=41	n=37

Figure 4. T-Lymphocyte profile. a. percentage of white blood cells with change over time, b. numbers of T-lymphocytes and change over time, placebo open bar) and treatment (solid bar) (mean +/- SEM) *P=0.023.

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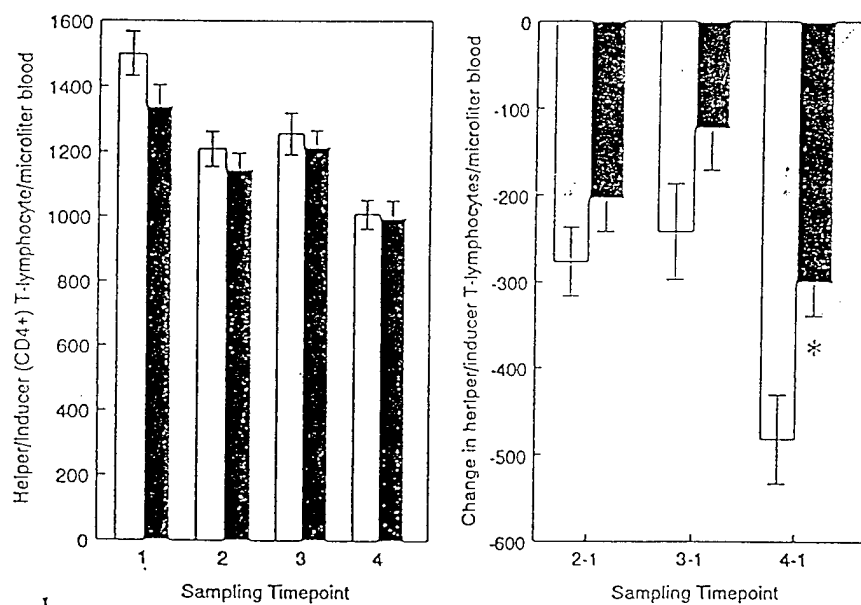
Figure 5. a. Percentage helper/inducer (CD4+) T-lymphocytes of blood and change over time. b. helper/inducer (CD4+) T-lymphocytes/microliter blood and change over time, placebo (open bar) and treatment (solid bar) (mean +/- SEM). *P=0.008.

In addition to the evaluation of the effects on the percent and numbers of important lymphocyte populations, we examined the ability of these lymphocytes to become activated (after mitogenic stimulation). We stimulated lymphocytes *ex vivo* to determine the helper T-cell type distribution (Th1, Th2) by measuring the cytokines that were secreted following stimulation. There was a decrease over time in the lymphocyte number and percentage of lymphocytes that could be activated (see Figure 6) as indicated by CD69 expression. There was no difference between treatment groups but within each group there was a statistically significant decrease in both the percentage of cells over time (visit 3-1 and visit 4-1, $P<0.001$ and Visit 4-1, $P<0.001$ for treatment and placebo groups, respectively) and for the number of activated lymphocytes following stimulation ($P<0.009$ for the change from baseline at visit 2, 3, and 4 for both groups).

There was a smaller decline of Th1 lymphocyte number ($P=0.029$) in the treatment group but no difference in the percentage between the two groups (see Figure 7). There was no difference between groups of Th2 lymphocytes in percent or numbers. Another functional assay was used to determine the percentage and number of cells that expressed CD40L, a T-lymphocyte activation marker that promotes cooperative B-lymphocyte function. There was a decrease over time in the number and percentage of lymphocytes that expressed CD40L. However, in the treatment group there was a reduced decline in CD40L lymphocyte number and percentage ($P=0.009$ and $P=0.024$, respectively).

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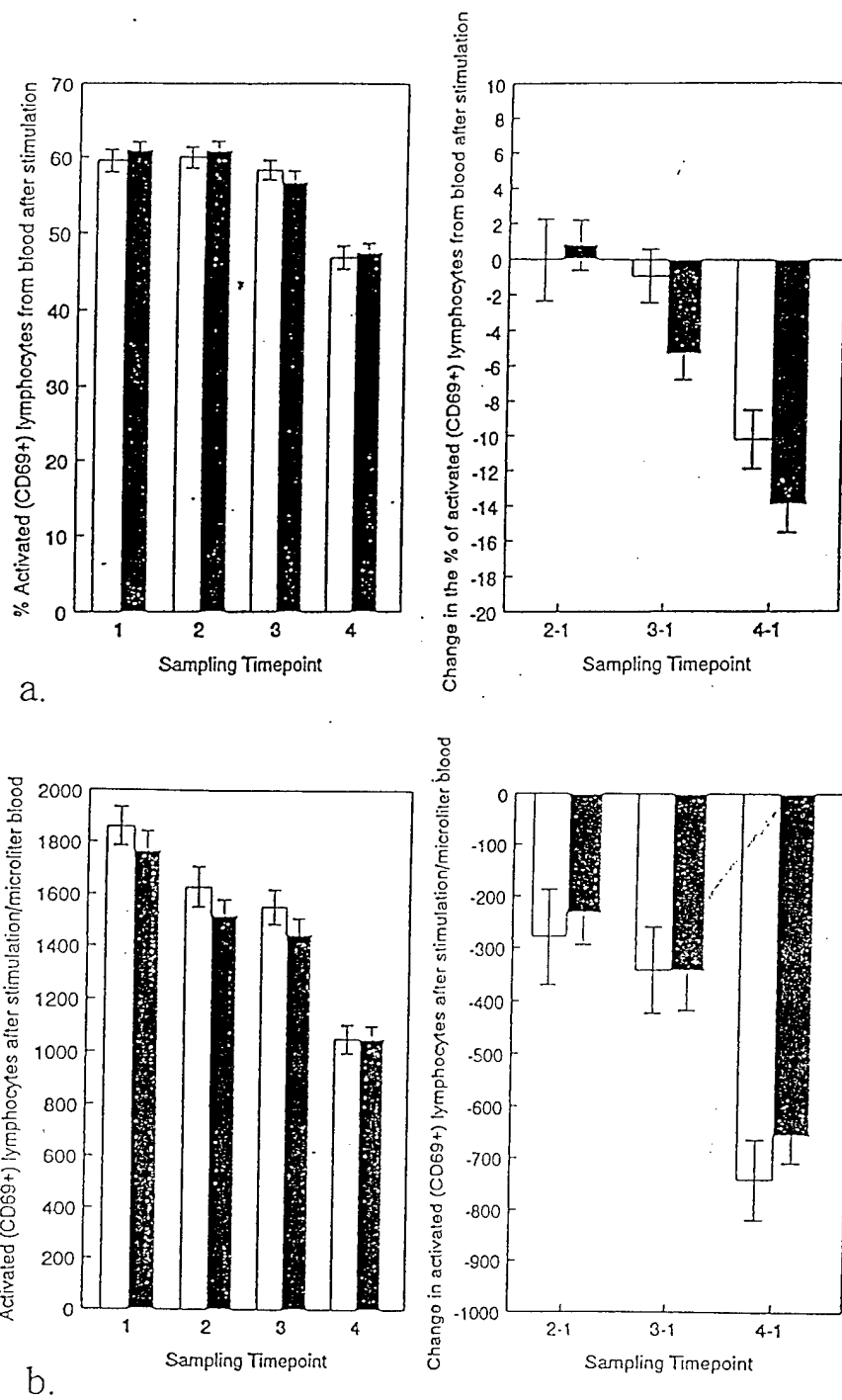
Figure 6. a. Percent activated (CD69+) lymphocytes from blood after stimulation with PHA for 4 hours and change over time. b. activated (CD69+) lymphocytes after stimulation/ microliter of blood and change over time, placebo (open bar) and treatment (solid bar) (mean +/- SEM).
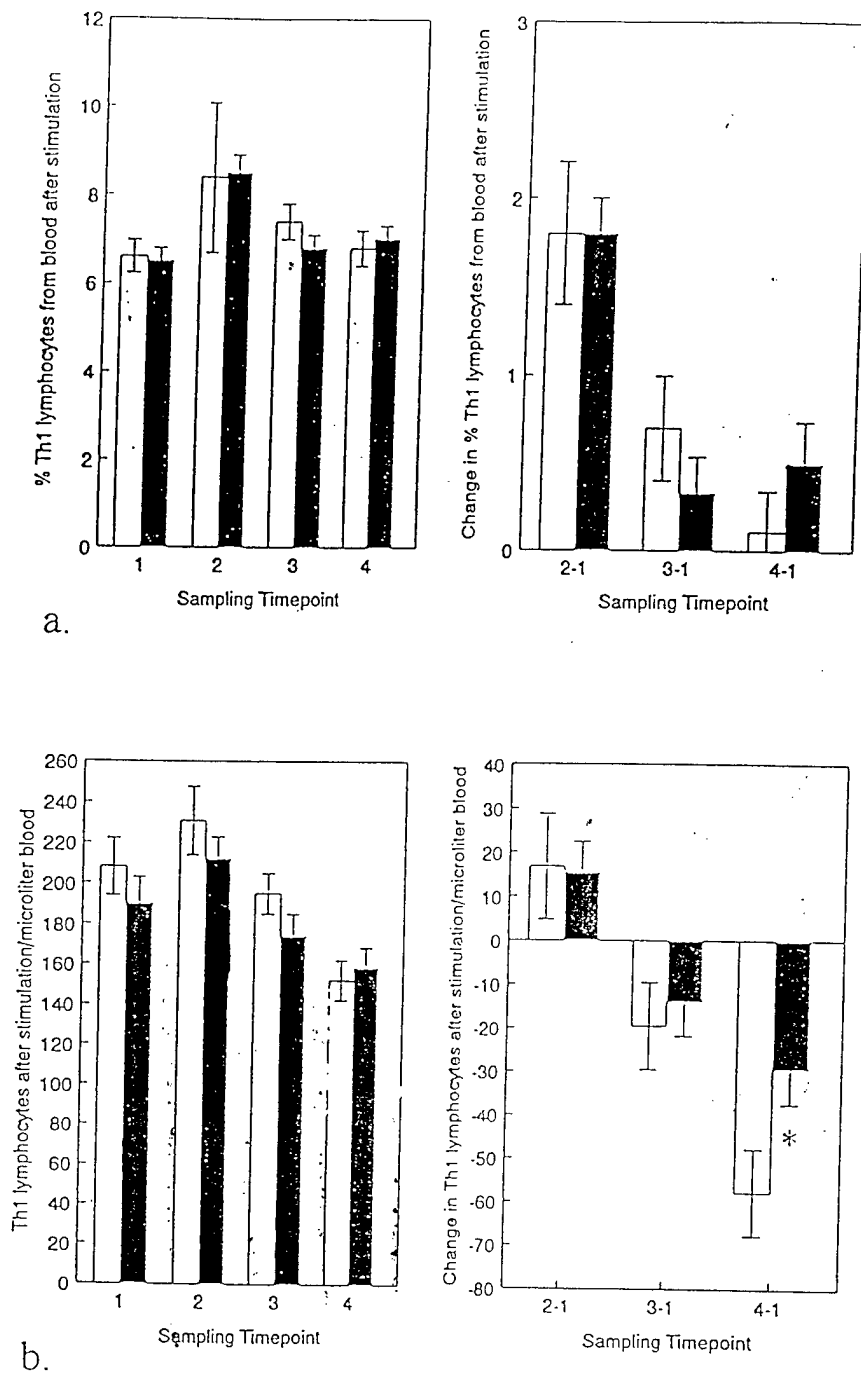
Figure 7. a. Percent Th1 (CD3+) lymphocytes after stimulation with PMA + ionomycin for 4 hours. Cells were then fixed, permeablized and stained with monoclonal antibodies, b. Th1 lymphocytes/uL after stimulation and change over time, placebo (open bar) and treatment (solid bar) (mean +/ -SEM, *P=.029).
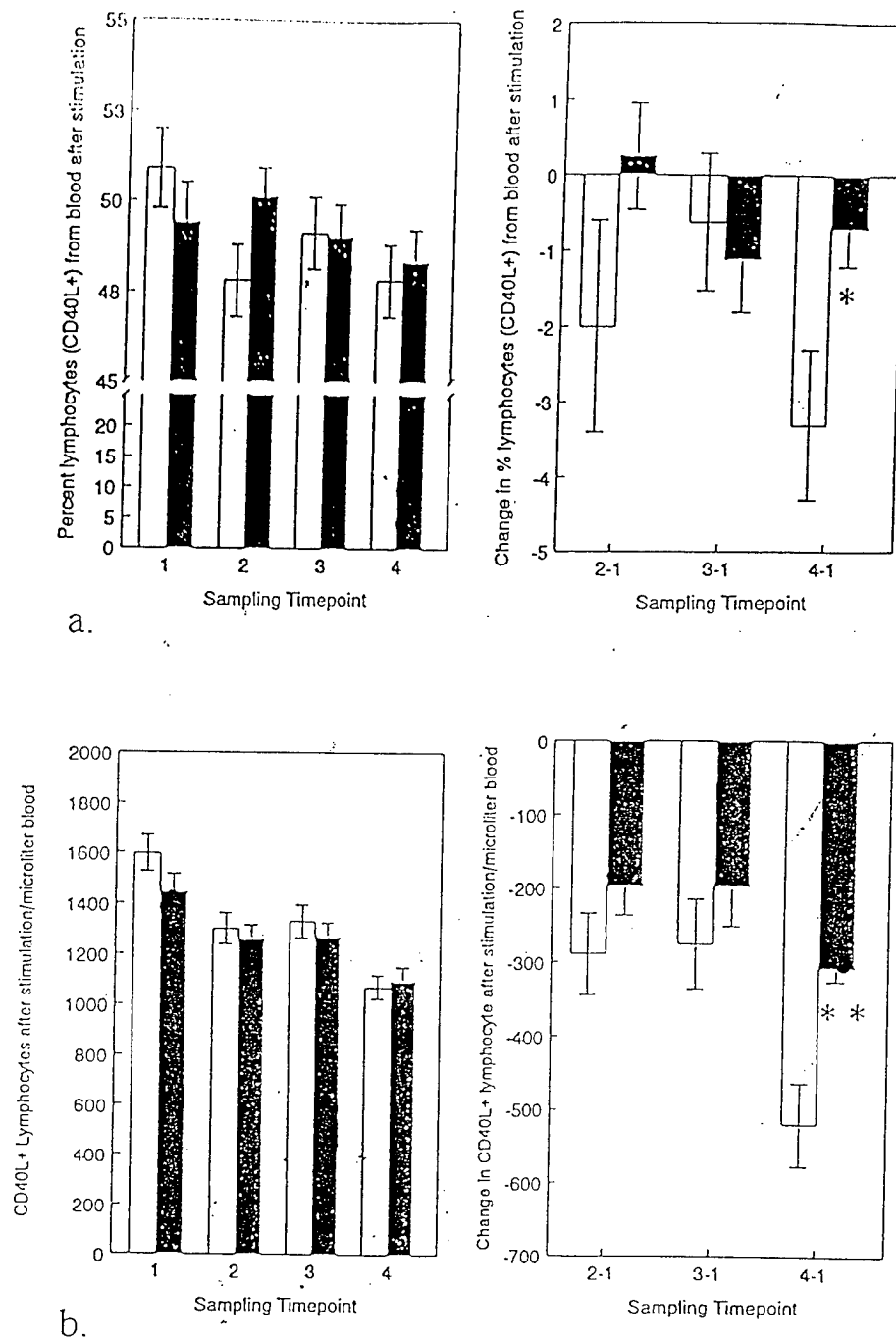
Figure 8. a. CD40L+ lymphocytes percent and change over time. b. number of CD40 L+ lymphocyte and change in the number of cells over time, placebo (open bar) and treatment (solid bar) (mean +/- SEM), *P = 0.024 and **P = 0.009.

There was a trend in the treatment group toward a reduced decrease in the number of B-Lymphocytes (Figure 9).
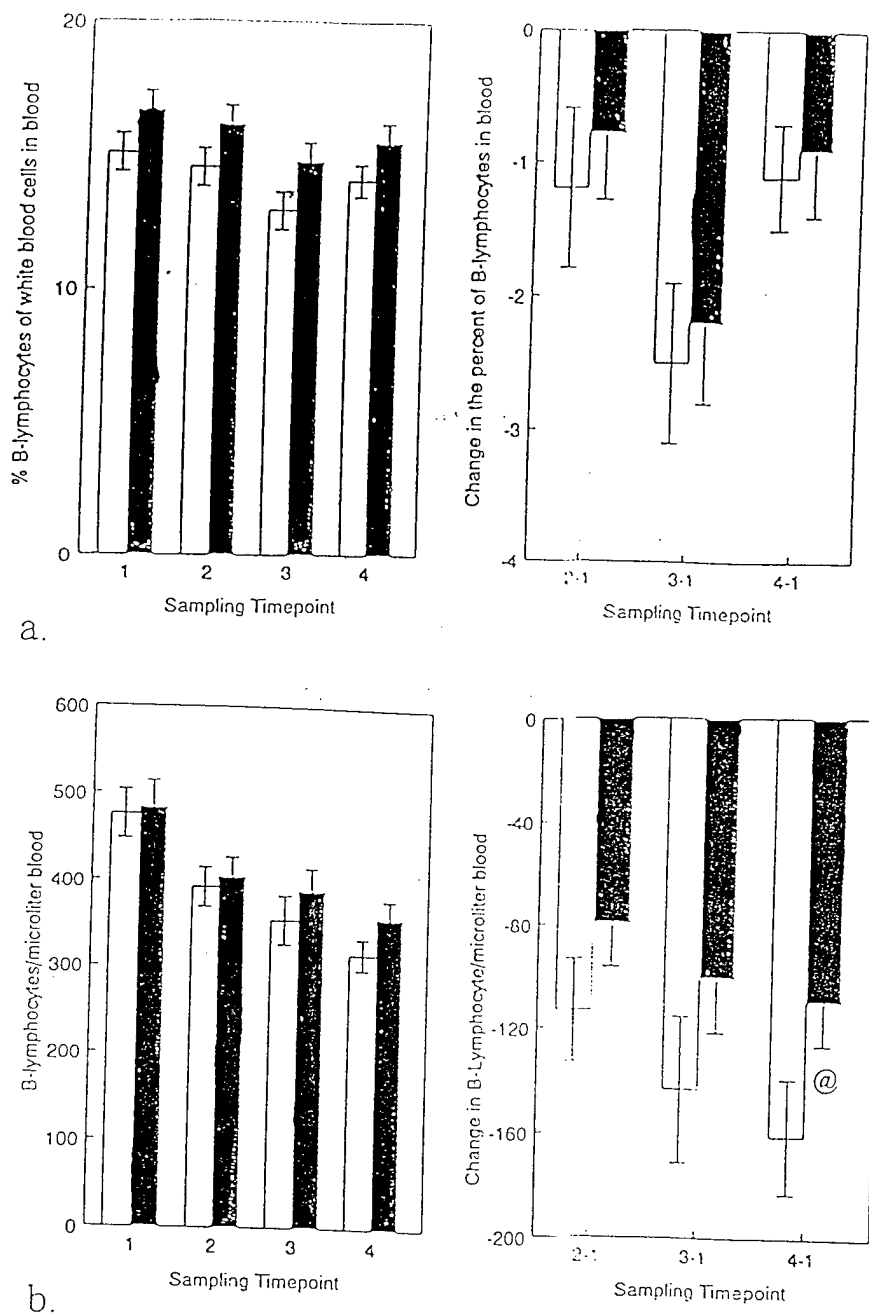
Figure 9. a. Percent B-lymphocytes (CD19&20+) and change over time. b. B-lymphocyte numbers and change over time, placebo (open bar) and treatment (solid bar) (mean +/- SEM), @P=0.058.

There was a loss of approximately 40-50% of B-lymphocyte numbers/µL (more dramatic in the placebo group, Figure, 10b). Further, there was a decreased ability of the B-lymphocytes (numbers) to become activated after stimulation. But, there was little change in the percent of CD69+ B-cells emphasizing the need to examine both percentages and number of lymphocytes.

a.

b.

Figure 10. Activated B-lymphocytes (CD69+) after stimulation with PHA for 4 hours. a. Percentage of lymphocytes and change over time, b. Change in B-lymphocyes/µL and change over time, placebo (open bar) and treatment (solid bar) (mean ± SEM).
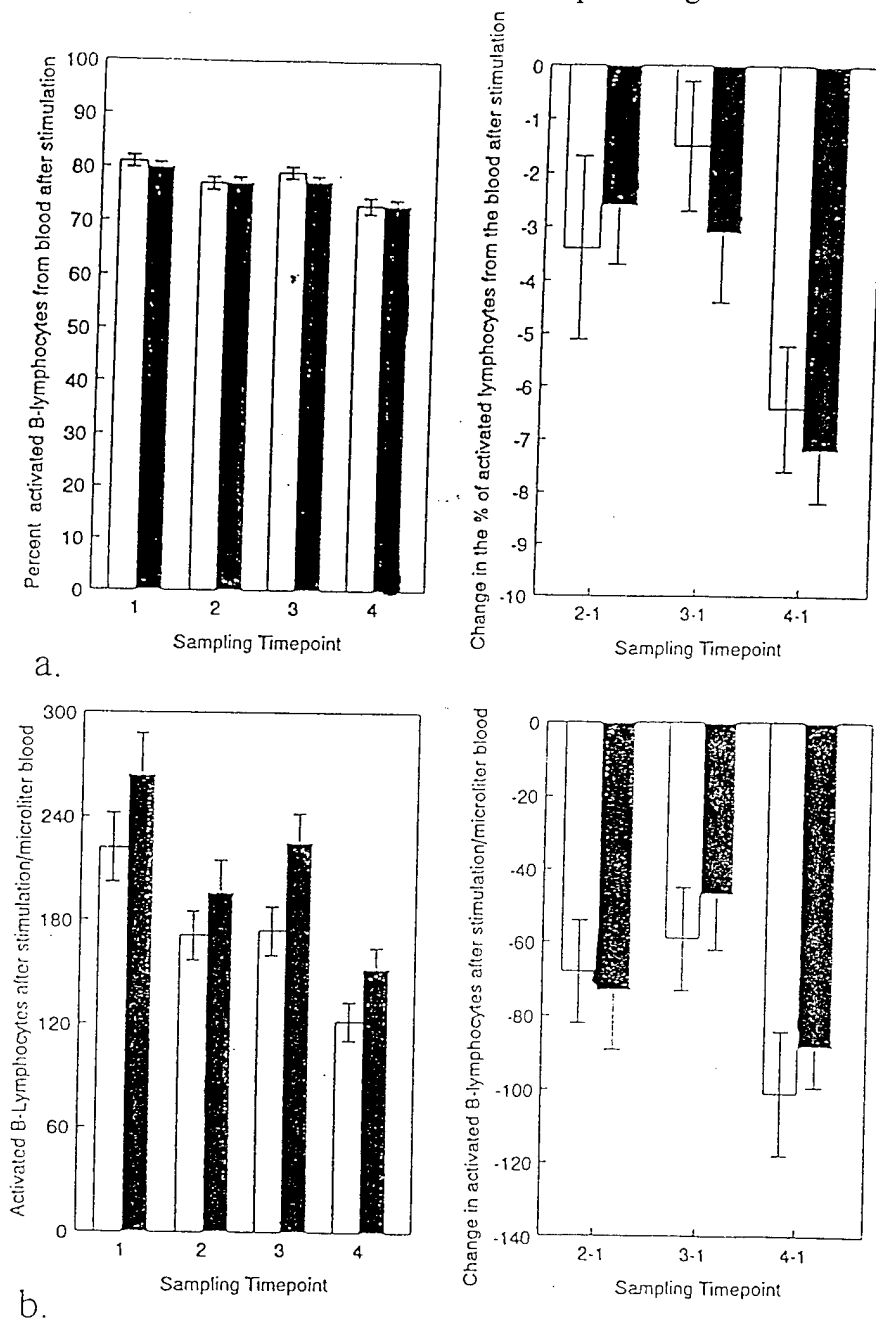
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There was a lower percentage of NK lymphocytes in both study groups expressing CD95 (an apoptosis marker), as compared to the reference group (even at baseline),once again indicating that the soldiers were showing signs of stress even before enrollment into the study. There was no significant difference between placebo and treatment in the NK cell numbers cells/µL (Table 9).

Table 9. Activated NK lymphocytes (TCR-CD16&56+\CD3-\CD95+) that would undergo apoptosis if they came in contact with the CD95 ligand.

	Visit 1	Visit 2	Visit 3	Visit 4
Cytotoxic NK Lymphocytes (Percent)				
Placebo	5.9	6.5 (0.89)	4 (-1.78)	6.1 (0.62)
Treatment	6.0	6.39 (-0.02)	4.58 (-1.96)	6.51 (-0.27)
Reference		10.2	12.8 (0.86)	12.1 (1.46)
Cytotoxic NK Lymphocytes ($x10^3$ cells/μL)				
Placebo	180.3	176.8 (1.1)	105.8 (-74.2)	137.1 (-35.5)
Treatment	171.0	157 (-29.2)	116.4 (-69.1)	141.7 (-48.3)
Reference		263.2	292.4 (31.1)	286.5 (11.9)

It is unlikely that the cell number changes are simply due to the effects of hydration, particularly since RT is so physically demanding. Hematocrit values indicated that there were no dramatic fluid shifts but hematocrit levels were lower in the treatment groups than the non-stressed group (Table 10). Values of serum creatinine and blood urea nitrogen, as well as urine specific gravity and serum osmolality, suggested no hypohydration or fluid compartment shifts, also reflecting the consistent efforts of training instructors to emphasize fluid replacement.

Table 10. Hematocrit of soldiers participating in Ranger Training. Soldiers consumed placebo or treatment bars and were compared to soldiers of similar age but not undergoing the demanding stress of Ranger Training (reference group). Values are means with change from baseline in parenthesis. Normal range 38-54% rbc

	Visit 1	Visit 2	Visit 3	Visit 4
Hematocrit (% rbc)				
Placebo Group	$40.3^{(60)}$	$39.5^{(55)}(-0.85)^{(51)}$	$42.4^{(54)} (1.9)^{(51)}$	$38.2^{(44)} (-2.2)^{(42)}$
Treatment Group	$40.7^{(62)}$	$39.8^{(65)}(-0.77)^{(54)}$	$42.1^{(65)} (1.4)^{(54)}$	$38.2^{(54)} (-2.3)^{(47)}$
Reference Group		$44.9^{(9)}$	$45.9^{(5)} (0.9)^{(5)}$	$46.9^{(8)} (1.8)^{(8)}$

SURVEY RESPONSES

Table 11 contains the number of subjects who responded to the question: Did the bar help you complete Ranger Training? The majority of subjects in both groups responded that the bars helped in completing RT, while more treatment than placebo subjects said that the bar helped them complete the Ranger Training course.

Table 11. Percentage of Soldiers responses to the question, Did the bar help complete Ranger Training? Number of responses divided by the total number who answered the question in parenthesis.

	No	Yes
Control Bar	33% (15/45)	67% (30/45)
Treatment Bar	24% (13/55)	76% (42/55)

The majority of the Rangers who consumed the bars responded that the size of the bar was adequate (65 grams). Approximately 30% of the subjects indicated that the bar was too small; however, many indicated that they preferred a larger bar because of their hunger. There were only a couple of subjects who indicated that the bars were too large (Table 12).

Table 12. Soldiers participating in Ranger Training who consumed two, 65 g bars for the entire
Ranger Training period were asked about the size of the bar they consumed.
Percentage of soldiers who responded with the number of responses divided by the
total number who answered the question in parentheses.

	Bar Size		
	Too Small	Just About Right	Too Large
Control Bar	29% (13/45)	69% (31/45)	2% (1/45)
Treatment Bar	35% (19/55)	64% (35/55)	2% (1/55)

The subjects were asked to respond to the question: Have you previously eaten other nutrition
or sports bars? Thirty three out of 45 (placebo group) and 30 out of 55 (treatment group)
responded that they had eaten other nutrition or sports bars. Those who answered the question
"yes" were asked to name the bars they have eaten. The following is a list of bars the soldiers had
eaten: Power Bars, Tiger Sport, Cliff Bar, Tiger Milk, VO2 Max, Metrix, granola bar, GNC
protein bar, Exceed bar and PB bar. Power Bars was the brand listed most frequently. The
subjects were then asked to list the bar that they like the best and compare that bar to the bar that
they consumed in the study (see Table 13). Eighty-seven percent of those who had consumed
other bars felt that the bars eaten in this study were better than the other bars while only 13% felt
that the bars consumed in this study were either about the same or slightly worse.

Table 13. Comparison of nutrition or sports bars which volunteers had previously consumed to
the bar they consumed in this study. Number of responses divided by the total number
who answered the question in parenthesis.

	Much Worse	Slightly worse	About the Same	Slightly Better	Much Better
Placebo Bar	0%	0%	6% (2/33)	18% (6/33)	76% (25/33)
Treatment Bar	0%	10% (3/30)	10% (3/30)	23% (7/30)	57% (17/30)
Total Responses	0%	5% (3/63)	8% (5/63)	21% (13/63)	67% (42/63)

The survey was also designed to have the subjects indicate their rating of the flavor, chocolate coating, sweetness, saltiness and texture (1-dislike very much to 7-like very much). We asked the subjects to respond on the bar's flavor, sweetness, and chewiness (very weak to very strong) with a comparison to their ideal bar. There was overall acceptance of both placebo and treatment bar for flavor, coating, sweetness and overall preference (Figure 11). The responses for both bars were toward optimal, i.e., like very much. However, it appeared that the subjects consuming the placebo bar rated it higher than those consuming the treatment bar for flavor, sweetness, and texture.
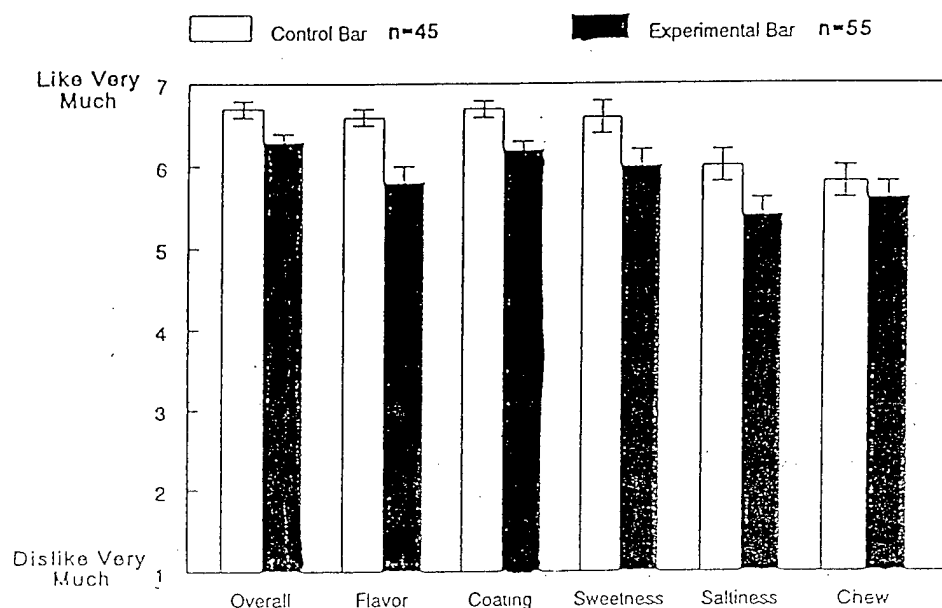

Figure 11. Response of subjects' preference to flavor, coating, sweetness, saltiness and chewiness.

Overall, the bars were well accepted and the soldiers perceived that the bars provided benefit during RT

DISCUSSION

We found that the stress of RT caused a number of consistent negative immunologic changes from baseline that correlated with decreases in general health. RT provides a model for the study of the effects of stress on immune status that can be characterized by a number of mechanisticlly oriented *in vivo* and *in vitro* immunological methods. Immune status in the RT model is also a sensitive indicator of the effects of nutritional intervention which may influence the negative immunological changes caused by stress. In the present study we found that the group consuming the bar containing antioxidants, structured lipid, indigestible carbohydrate, zinc, copper, selenium, taurine, carnitine and other vitamins and minerals attenuated the decrease in several measures of

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immune status. The group consuming the treatment bar had a reduced decline in important immune cells: T-lymphocytes, helper/inducer T-lymphocytes, Th1 lymphocytes and lymphocyte function as determined by CD40L expression. There were no differences between groups in response to vaccination and delayed-type hypersensitivity although they were both depressed as compared to the non-stressed reference group. There were other markers, e.g. body weight changes and maintenance of B-lymphocytes, that although not statistically significant between the placebo and treatment groups, indicate a benefit of the treatment bar. Both nutrition bars were well accepted and the majority of subjects indicated that the bar helped them complete RT.

Soldiers under combat or stressful conditions have been shown to be at risk for infectious diseases (31). For example, at the Naval Hospital, Camp Pendleton, CA, it was estimated that the hospitalization rate for pneumonia was 38 times that of the USN and USMC in general (33). Similarly, diarrheal disease was a major threat and concern to US military forces during Operation Desert Shield (40). Soldiers typically find themselves deployed to living conditions that place them at risk to infections. Nutritional intervention may have the potential to maintain or enhance immune function during stressful conditions.

Several researchers have demonstrated in a variety of animal and human studies the immunological effects of stress. Cellular immunity (DTH) was assessed in cosmonauts during long duration space flight, and it was found that responsiveness to the DTH test decreased below the "warning level" (29). The immunological changes that occurred during space flight returned to normal levels after landing. Similar findings were shown in chronic stress with cutaneous DTH and cell mediated immunity in mice (20). In the current study we found that the antibody response to an antigen delivered in a vaccine was approximately 30% lower in soldiers undergoing RT as compared to the reference, non-stressed group. Further, approximately 20% of the subjects did not attain a protective antibody titer after being vaccinated during stressful training. The current study was conducted to determine the impact of stress on a primary immune response with the vaccine against hepatitis A, which is a potent immunogen. Other researchers have found that chronic stress alters the immune response to vaccination (49). This study identified an immune response to influenza virus vaccine (secondary response) in 38% of caregivers while 60% of non-caregivers responded. A similar finding of decreased antibody response to hepatitis B virus (primary) vaccine was also noted in a study of young, healthy medical students under academic stress (28). Suppressed response to a vaccine appears to be a metric of immunologic stress. Whether this can be minimized by a nutritional formula remains to be determined.

Our inability to document clearly the impact of a nutritional supplement vaccine response may have been related to the reduced sample size. We calculated the sample size necessary to determine a statistically significant difference. If we use the reference group as an example of a positive effect of nutritional intervention and the placebo group's responses as typical of the RT course, between 199 - 231 subjects per group would be required with a significance level of 0.05 and a power of 80%. This indicates that we were statistically under-powered to determine differences between products based on vaccine responses. Despite this drawback there were

cellular changes that seemed to be attenuated in the treatment group.

Previous studies of soldiers participating in RT reported a typical weight loss of 20 - 30 pounds. In this study there was an average weight gain in the treatment group which was slightly greater (P 0.068) than that of the placebo group. This indicated that the immune suppression was certainly not the result of an energy deficiency. It also indicated that the additional energy provided by the bars minimized weight loss, but did not prevent a decrease in immune function.

Few studies have addressed the immunological impact of chronic stress in humans as in this study, especially large scale flow cytometry studies and nutritional intervention trials. Changes in immunologic function that place soldiers at increased risk of infection are: 1) a decrease in white blood cells, monocytes, the number of T-lymphocytes, number and ability of the lymphocytes to respond (stimulated CD69 expression, CD69 intensity); 2) percent and number of B-lymphocytes and ability to become activated, and 3) the decreased ability of NK cells to become activated. Dekaris et al. (18) found similar immunologic changes in lymphocyte subpopulations in prisoners of war, and stated "...immune reactivity could be down regulated to the point as to provide inadequate protection to infections or to cause autoimmunity (p. 599)." We found that the consumption of the treatment bars attenuated the decline of specific lymphocyte populations and provided an indication that the nutritional components helped maintain immune responses under stressful conditions. For example, there was an attenuated decline from baseline in the number of total T-lymophcytes, helper/inducer T-lymphocytes, memory helper/inducer T-lymphocytes, and Th1 lymphocytes after stimulation, CD40L+ lymphocytes (co-stimulatory ligand), and number of B-lymphocytes. Cell-to-cell interactions play a critical role in an effective immune response. The findings of this study suggest that the treatment bar maintained cellular components that are required and regulate immune responses. One component of this is adequate CD4+ cells and expression of CD40 and its ligand (CD40L). Impairment of this interaction dampens the immune responsiveness. Furthermore, this interaction is necessary for B-cell differentiation and antibody class switching as well as activation of macrophages to produce cytokines. Successful control and interaction of CD40L on T-cells are therefore important for effective immune function (35), and the group consuming the treatment bar experienced a reduced decline in these lymphocytes.

It was interesting that the treatment group maintained its weight more closely than the placebo group. During physical exercise, muscle cells rely on both carbohydrates and lipids as energy sources but the utilization is dictated by intensity and duration. Fat is more energy dense than CHO, and under resting conditions or low intensity exercise, fatty acids contribute the majority of energy needs. When exercise intensity is increased, there is a shift to CHO utilization yet very little CHO (glycogen) can be stored in the muscle thus limiting high power output during endurance or long term exercise. Further, glycogen depletion is correlated with fatigue (7). It has also been found by stable-isotope technologies that as much as 20-25% of energy expenditure may be derived from muscle triglyceride during prolonged exercise at moderate intensity (73). MCT have been studied during short periods of physical exercise; however, they have not demonstrated any advantages, presumably because they are metabolized quickly by the liver. Included in the treatment product of this study was a structured lipid, designed to increase the

absorption of the MCFA into the lymphatic and circulatory systems, to provide an energy source before metabolism by the liver. Furthermore, we attempted to provide a unique and balanced combination of fats and carbohydrates. Van Zyl et al. (84) studied a sports drinks containing MCT, CHO + MCT, or CHO alone. They found that there was a significant improvement in performance in the group that received the CHO + MCT over the CHO or MCT alone. There have not been human studies investigating the effects of structured lipids on performance and this provides a unique strategy and opportunity for future research.

Supplementation as a means to minimize or prevent immune changes and protect individuals who are undergoing physical stress has been studied to a limited extent. For example, Peters et al, (68) supplemented the diets of ultramarathon runners for 21 days prior to an ultra-marathon with either 600 mg vitamin C/day or a placebo. They then assessed the incidence of symptoms of upper respiratory tract infection for the 14 days following the race. Sixty-eight percent of the runners who consumed the placebo reported upper respiratory tract infections while only 33 percent of those who consumed the vitamin C. Vitamin C is found in high concentrations in leukocytes and its concentration decreases rapidly during infections suggesting that it plays an important role in immune response (82). It has also been shown that physical activity raises oxygen demand several fold, thus causing an increase in the formation of oxygen radical species (1, 27). As oxygen radicals are formed and accumulate, they adversely affect immune function (3). Theoretically, providing antioxidants in sufficient amounts during exercise-induced oxidative stress may reduce immune dysregulation (91). Furthermore, several reviews (11, 15) have emphasized the importance of dietary components, such as antioxidants, on multiple aspects of the immune response and immune system, including the several antioxidants assessed in the current study.

Decreased DTH response or anergy reflects increased risk of morbidity and mortality, especially in the elderly (88). The extent of the DTH response has also been used as a marker of immune responsiveness (56). In the current study there was no difference in DTH or antibody responses between the placebo and treatment groups; however, it appeared that the soldiers in this study were not as physically stressed as previous RT classes (average total DTH induration of 6.3 mm total compared to 4.4 mm in previous RT classes). This was also different from the previous study conducted in Special Forces Selection and Assessment School (21 days duration) in which the treatment (same formulation but in liquid form) minimized the decrease in DTH anergy, and there was a trend toward a greater response 9.8 vs 7.8 mm (P=0.07) as compared to the placebo group (48). In terms of the value of DTH in predicting morbidity and mortality, it has been utilized in many scenarios (ie. elderly and hospitalized). For example, Fuller et al. (26) studied a model of immune suppression (chronic exposure to ultraviolet light) and the effects of β- carotene supplementation on the DTH response in young male volunteers (similar in age to those in this study). Subjects were fed a diet low in carotenoid content and then fed a β-carotene supplement or a placebo during 16 days of UV exposure. The DTH response was suppressed in the UV-exposed group receiving the placebo, while the β-carotene supplement returned the incidence and intensity of the DTH response to normal. Other nutrients included in the current treatment bar have been shown to affect DTH responses, i.e., zinc (25), selenium (8), and vitamin E (56).

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The increase of anergy in soldiers participating in RT may have predictive value of the risk of infection, and the effects of nutritional intervention on the DTH responses provide a useful model for further investigating these variables.

Our data demonstrated that the soldiers readily accepted both the placebo and treatment bars during RT. Many soldiers indicated that the bars helped in completing RT (67% and 76% in the placebo and treatment groups, respectively). The soldiers also indicated that the size of the bar (65 grams) was adequate while fewer soldiers felt that the bar was too small. Lastly, the soldiers were asked to rate each bar on a hedonic (1-disliked it very much to 7-liked very much) of flavor coating, sweetness, saltiness and chewiness. Both placebo and treatment bars were well accepted (average score for both bars was between 6 and 7). Perhaps the high concentration of vitamins and minerals could potentially decrease the overall acceptance of the treatment bars. Future work will be done on optimizing the flavor and other characteristics, but overall the bars were well accepted and there was a strong perception that the bars helped the trainees complete the rigorous scenario.

CONCLUSION

Overall this study shows that RT induces measurable, negative immunological changes which are attenuated but not eliminated by a novel nutritional bar which was popular and well tolerated by the trainees. Further studies with improved formulations may provide even better immunological improvements and are recommended. In this study, soldiers completing RT experienced several immunological changes but the treatment product lessened the decline of important immune cells (T-, CD4+, CD40L+, and Th1 lymphocytes) indicating that they may be at a lower risk of infection. There was also a trend toward greater body weight maintenance in the treatment group and a smaller loss of body weight (in both groups) as compared to previous Ranger classes. It appears that providing adequate energy reduced the stress of the course in both the placebo and treatment groups. Furthermore, since the soldiers in both groups gained weight it made it more difficult to detect immunological differences; however, differences were detected in cellular changes. There was no difference between the placebo and treatment groups with respect to response to hepatitis vaccine and DTH. Nevertheless, future studies should be conducted with other vaccines (less immunologically potent than hepatitis A, i.e. influenza or hepatitis B) to determine whether the treatment bar containing antioxidants, structured lipid (MCT/canola), indigestible carbohydrate, minerals and other nutrients, known to affect immune function, minimizes immune suppression caused from stress. Results in this study appear to show that the formulation used affected some key lymphocyte populations. The treatment bar was well accepted by the soldiers who consumed two bars per day for approximately sixty days. The treatment bar should also be studied in a sports nutrition environment and evaluated for its influence on performance. Other immune compromised populations (e.g. elderly) should be tested using either the liquid or bar formulations to confirm its immunologic benefit.

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